

Dilutions of the fusion protein test material (50 μ l) is added in triplicate to microtiter wells. Test samples (of the protein of interest) are added for final dilution of 1:4; rhuIL-2 (R&D Systems, Minneapolis, MN, catalog number 202-IL) is added to a final concentration of 1 μ g/ml; anti-CD4 mAb (R&D Systems, clone 34930.11, catalog number MAB379) is added to 5 a final concentration of 10 μ g/ml. Cells are cultured for 7-8 days at 37°C in 5% CO₂, and 1 μ C of [³H] thymidine is added to wells for the last 16 hrs of culture. Cells are harvested and thymidine incorporation determined using a Packard TopCount. Data is expressed as the mean and standard deviation of triplicate determinations.

10 Samples of the fusion protein of interest are screened in separate experiments and compared to the negative control treatment, anti-CD4 mAb, which inhibits proliferation of lymphocytes and the positive control treatment, IL-2 (either as recombinant material or supernatant), which enhances proliferation of lymphocytes.

Example 43: Assays for Protease Activity

15 The following assay may be used to assess protease activity of an albumin fusion protein of the invention.

20 Gelatin and casein zymography are performed essentially as described (Heusen et al., *Anal. Biochem.*, 102:196-202 (1980); Wilson et al., *Journal of Urology*, 149:653-658 (1993)). Samples are run on 10% polyacryamide/0.1% SDS gels containing 1% gelatin or casein, soaked in 2.5% triton at room temperature for 1 hour, and in 0.1M glycine, pH 8.3 at 37°C 5 to 16 hours. After staining in amido black areas of proteolysis appear as clear areas against the blue-black background. Trypsin (Sigma T8642) is used as a positive control.

25 Protease activity is also determined by monitoring the cleavage of n-a-benzoyl-L-arginine ethyl ester (BAEE) (Sigma B-4500. Reactions are set up in (25mM NaPO₄, 1mM EDTA, and 1mM BAEE), pH 7.5. Samples are added and the change in adsorbance at 260nm is monitored on the Beckman DU-6 spectrophotometer in the time-drive mode. Trypsin is used as a positive control.

30 Additional assays based upon the release of acid-soluble peptides from casein or hemoglobin measured as adsorbance at 280 nm or colorimetrically using the Folin method are performed as described in Bergmeyer, et al., *Methods of Enzymatic Analysis*, 5 (1984). Other assays involve the solubilization of chromogenic substrates (Ward, *Applied Science*, 251-317 (1983)).

Example 44: Identifying Serine Protease Substrate Specificity

35 Methods known in the art or described herein may be used to determine the substrate

specificity of the albumin fusion proteins of the present invention having serine protease activity. A preferred method of determining substrate specificity is by the use of positional scanning synthetic combinatorial libraries as described in GB 2 324 529 (incorporated herein in its entirety).

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Example 45: Ligand Binding Assays

The following assay may be used to assess ligand binding activity of an albumin fusion protein of the invention.

Ligand binding assays provide a direct method for ascertaining receptor pharmacology and are adaptable to a high throughput format. The purified ligand for an albumin fusion protein of the invention is radiolabeled to high specific activity (50-2000 Ci/mmol) for binding studies. A determination is then made that the process of radiolabeling does not diminish the activity of the ligand towards the fusion protein. Assay conditions for buffers, ions, pH and other modulators such as nucleotides are optimized to establish a workable signal to noise ratio for both membrane and whole cell polypeptide sources. For these assays, specific polypeptide binding is defined as total associated radioactivity minus the radioactivity measured in the presence of an excess of unlabeled competing ligand. Where possible, more than one competing ligand is used to define residual nonspecific binding.

20 *Example 46: Functional Assay in Xenopus Oocytes*

Capped RNA transcripts from linearized plasmid templates encoding an albumin fusion protein of the invention is synthesized in vitro with RNA polymerases in accordance with standard procedures. In vitro transcripts are suspended in water at a final concentration of 0.2 mg/ml. Ovarian lobes are removed from adult female toads, Stage V defolliculated oocytes are obtained, and RNA transcripts (10 ng/oocyte) are injected in a 50 nl bolus using a microinjection apparatus. Two electrode voltage clamps are used to measure the currents from individual *Xenopus oocytes* in response fusion protein and polypeptide agonist exposure. Recordings are made in Ca²⁺ free Barth's medium at room temperature. The Xenopus system can be used to screen known ligands and tissue/cell extracts for activating ligands.

30 *Example 47: Microphysiometric Assays*

Activation of a wide variety of secondary messenger systems results in extrusion of small amounts of acid from a cell. The acid formed is largely as a result of the increased metabolic activity required to fuel the intracellular signaling process. The pH changes in the media surrounding the cell are very small but are detectable by the CYTOSENSOR microphysiometer (Molecular Devices Ltd., Menlo Park, Calif.). The CYTOSENSOR is thus

capable of detecting the ability of an albumin fusion protein of the invention to activate secondary messengers that are coupled to an energy utilizing intracellular signaling pathway.

Example 48: Extract/Cell Supernatant Screening

5 A large number of mammalian receptors exist for which there remains, as yet, no cognate activating ligand (agonist). Thus, active ligands for these receptors may not be included within the ligands banks as identified to date. Accordingly, the albumin fusion proteins of the invention can also be functionally screened (using calcium, cAMP, microphysiometer, oocyte electrophysiology, etc., functional screens) against tissue extracts
10 to identify natural ligands for the Therapeutic protein portion and/or albumin protein portion of an albumin fusion protein of the invention. Extracts that produce positive functional responses can be sequentially subfractionated until an activating ligand is isolated and identified.

15 *Example 49: ATP-binding assay*

The following assay may be used to assess ATP-binding activity of fusion proteins of the invention.

ATP-binding activity of an albumin fusion protein of the invention may be detected using the ATP-binding assay described in U.S. Patent 5,858,719, which is herein incorporated by reference in its entirety. Briefly, ATP-binding to an albumin fusion protein of the invention is measured via photoaffinity labeling with 8-azido-ATP in a competition assay. Reaction mixtures containing 1 mg/ml of ABC transport protein are incubated with varying concentrations of ATP, or the non-hydrolyzable ATP analog adenylyl-5'-imidodiphosphate for 10 minutes at 4°C. A mixture of 8-azido-ATP (Sigma Chem. Corp., St. Louis, MO.) plus 8-
25 azido-ATP (32 P-ATP) (5 mCi/ μ mol, ICN, Irvine CA.) is added to a final concentration of 100 μ M and 0.5 ml aliquots are placed in the wells of a porcelain spot plate on ice. The plate is irradiated using a short wave 254 nm UV lamp at a distance of 2.5 cm from the plate for two one-minute intervals with a one-minute cooling interval in between. The reaction is stopped by addition of dithiothreitol to a final concentration of 2mM. The incubations are subjected to
30 SDS-PAGE electrophoresis, dried, and autoradiographed. Protein bands corresponding to the albumin fusion proteins of the invention are excised, and the radioactivity quantified. A decrease in radioactivity with increasing ATP or adenylyl-5'-imidodiphosphate provides a measure of ATP affinity to the fusion protein.

35 *Example 50: Phosphorylation Assay*

In order to assay for phosphorylation activity of an albumin fusion protein of the invention, a phosphorylation assay as described in U.S. Patent 5,958,405 (which is herein

incorporated by reference) is utilized. Briefly, phosphorylation activity may be measured by phosphorylation of a protein substrate using gamma-labeled ^{32}P -ATP and quantitation of the incorporated radioactivity using a gamma radioisotope counter. The fusion protein of the invention is incubated with the protein substrate, ^{32}P -ATP, and a kinase buffer. The ^{32}P incorporated into the substrate is then separated from free ^{32}P -ATP by electrophoresis, and the incorporated ^{32}P is counted and compared to a negative control. Radioactivity counts above the negative control are indicative of phosphorylation activity of the fusion protein.

10 ***Example 51: Detection of Phosphorylation Activity (Activation) of an
Albumin Fusion Protein of the Invention in the Presence of Polypeptide
Ligands***

Methods known in the art or described herein may be used to determine the phosphorylation activity of an albumin fusion protein of the invention. A preferred method of determining phosphorylation activity is by the use of the tyrosine phosphorylation assay as 15 described in US 5,817,471 (incorporated herein by reference).

20 ***Example 52: Identification Of Signal Transduction Proteins That
Interact With An albumin fusion protein Of The Present Invention***

Albumin fusion proteins of the invention may serve as research tools for the 25 identification, characterization and purification of signal transduction pathway proteins or receptor proteins. Briefly, a labeled fusion protein of the invention is useful as a reagent for the purification of molecules with which it interacts. In one embodiment of affinity purification, an albumin fusion protein of the invention is covalently coupled to a chromatography column. Cell-free extract derived from putative target cells, such as carcinoma tissues, is passed over the column, and molecules with appropriate affinity bind to the albumin fusion protein. The protein complex is recovered from the column, dissociated, and the recovered molecule subjected to N-terminal protein sequencing. This amino acid sequence is then used to identify the captured molecule or to design degenerate 30 oligonucleotide probes for cloning the relevant gene from an appropriate cDNA library.

Example 53: IL-6 Bioassay

A variety of assays are known in the art for testing the proliferative effects of an 35 albumin fusion protein of the invention. For example, one such assay is the IL-6 Bioassay as described by Marz *et al.* (*Proc. Natl. Acad. Sci., U.S.A.*, 95:3251-56 (1998), which is herein incorporated by reference). After 68 hrs. at 37°C, the number of viable cells is measured by adding the tetrazolium salt thiazolyl blue (MTT) and incubating for a further 4 hrs. at 37°C. B9 cells are lysed by SDS and optical density is measured at 570 nm. Controls

containing IL-6 (positive) and no cytokine (negative) are Briefly, IL-6 dependent B9 murine cells are washed three times in IL-6 free medium and plated at a concentration of 5,000 cells per well in 50 μ l, and 50 μ l of fusion protein of the invention is added. utilized. Enhanced proliferation in the test sample(s) (containing an albumin fusion protein of the invention) 5 relative to the negative control is indicative of proliferative effects mediated by the fusion protein.

Example 54: Support of Chicken Embryo Neuron Survival

To test whether sympathetic neuronal cell viability is supported by an albumin fusion 10 protein of the invention, the chicken embryo neuronal survival assay of Senaldi *et al* may be utilized (*Proc. Natl. Acad. Sci., U.S.A.*, 96:11458-63 (1998), which is herein incorporated by reference). Briefly, motor and sympathetic neurons are isolated from chicken embryos, resuspended in L15 medium (with 10% FCS, glucose, sodium selenite, progesterone, conalbumin, putrescine, and insulin; Life Technologies, Rockville, MD.) and Dulbecco's 15 modified Eagles medium [with 10% FCS, glutamine, penicillin, and 25 mM Hepes buffer (pH 7.2); Life Technologies, Rockville, MD.], respectively, and incubated at 37°C in 5% CO₂ in the presence of different concentrations of the purified fusion protein of the invention, as well as a negative control lacking any cytokine. After 3 days, neuron survival is determined by evaluation of cellular morphology, and through the use of the colorimetric assay of 20 Mosmann (Mosmann, T., *J. Immunol. Methods*, 65:55-63 (1983)). Enhanced neuronal cell viability as compared to the controls lacking cytokine is indicative of the ability of the albumin fusion protein to enhance the survival of neuronal cells.

Example 55: Assay for Phosphatase Activity

25 The following assay may be used to assess serine/threonine phosphatase (PTPase) activity of an albumin fusion protein of the invention.

In order to assay for serine/threonine phosphatase (PTPase) activity, assays can be utilized which are widely known to those skilled in the art. For example, the serine/threonine phosphatase (PSPase) activity of an albumin fusion protein of the invention may be measured 30 using a PSPase assay kit from New England Biolabs, Inc. Myelin basic protein (MyBP), a substrate for PSPase, is phosphorylated on serine and threonine residues with cAMP-dependent Protein Kinase in the presence of [³²P]ATP. Protein serine/threonine phosphatase activity is then determined by measuring the release of inorganic phosphate from 32P-labeled MyBP.

Example 56: Interaction of Serine/Threonine Phosphatases with other Proteins

Fusion protein of the invention having serine/threonine phosphatase activity (e.g., as determined in Example 55) are useful, for example, as research tools for the identification, 5 characterization and purification of additional interacting proteins or receptor proteins, or other signal transduction pathway proteins. Briefly, a labeled fusion protein of the invention is useful as a reagent for the purification of molecules with which it interacts. In one embodiment of affinity purification, an albumin fusion protein of the invention is covalently coupled to a chromatography column. Cell-free extract derived from putative target cells, such 10 as neural or liver cells, is passed over the column, and molecules with appropriate affinity bind to the fusion protein. The fusion protein -complex is recovered from the column, dissociated, and the recovered molecule subjected to N-terminal protein sequencing. This amino acid sequence is then used to identify the captured molecule or to design degenerate oligonucleotide probes for cloning the relevant gene from an appropriate cDNA library.

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Example 57: Assaying for Heparanase Activity

There a numerous assays known in the art that may be employed to assay for heparanase activity of an albumin fusion protein of the invention. In one example, heparanase activity of an albumin fusion protein of the invention, is assayed as described by Vlodavsky 20 et al., (Vlodavsky et al., Nat. Med., 5:793-802 (1999)). Briefly, cell lysates, conditioned media, intact cells (1×10^6 cells per 35-mm dish), cell culture supernatant, or purified fusion protein are incubated for 18 hrs at 37°C, pH 6.2-6.6, with 35 S-labeled ECM or soluble ECM derived peak I proteoglycans. The incubation medium is centrifuged and the supernatant is analyzed by gel filtration on a Sepharose CL-6B column (0.9 x 30 cm). Fractions are eluted 25 with PBS and their radioactivity is measured. Degradation fragments of heparan sulfate side chains are eluted from Sepharose 6B at $0.5 < K_{av} < 0.8$ (peak II). Each experiment is done at least three times. Degradation fragments corresponding to "peak II," as described by Vlodavsky et al., is indicative of the activity of an albumin fusion protein of the invention in cleaving heparan sulfate.

30

Example 58: Immobilization of biomolecules

This example provides a method for the stabilization of an albumin fusion protein of the invention in non-host cell lipid bilayer constructs (see, e.g., Bieri et al., Nature Biotech 17:1105-1108 (1999), hereby incorporated by reference in its entirety herein) which can be 35 adapted for the study of fusion proteins of the invention in the various functional assays described above. Briefly, carbohydrate-specific chemistry for biotinylation is used to confine a biotin tag to an albumin fusion protein of the invention, thus allowing uniform orientation

upon immobilization. A 50uM solution of an albumin fusion protein of the invention in washed membranes is incubated with 20 mM NaIO4 and 1.5 mg/ml (4mM) BACH or 2 mg/ml (7.5mM) biotin-hydrazide for 1 hr at room temperature (reaction volume, 150ul). Then the sample is dialyzed (Pierce Slidealizer Cassett, 10 kDa cutoff; Pierce Chemical Co., 5 Rockford IL) at 4C first for 5 h, exchanging the buffer after each hour, and finally for 12 h against 500 ml buffer R (0.15 M NaCl, 1 mM MgCl2, 10 mM sodium phosphate, pH7). Just before addition into a cuvette, the sample is diluted 1:5 in buffer ROG50 (Buffer R supplemented with 50 mM octylglucoside).

10 *Example 59: Assays for Metalloproteinase Activity*

Metalloproteinases are peptide hydrolases which use metal ions, such as Zn²⁺, as the catalytic mechanism. Metalloproteinase activity of an albumin fusion protein of the present invention can be assayed according to methods known in the art. The following exemplary methods are provided:

15 *Proteolysis of alpha-2-macroglobulin*

To confirm protease activity, a purified fusion protein of the invention is mixed with the substrate alpha-2-macroglobulin (0.2 unit/ml; Boehringer Mannheim, Germany) in 1x assay buffer (50 mM HEPES, pH 7.5, 0.2 M NaCl, 10 mM CaCl₂, 25 μM ZnCl₂ and 0.05% Brij-35) and incubated at 37°C for 1-5 days. Trypsin is used as positive control. Negative 20 controls contain only alpha-2-macroglobulin in assay buffer. The samples are collected and boiled in SDS-PAGE sample buffer containing 5% 2-mercaptoethanol for 5-min, then loaded onto 8% SDS-polyacrylamide gel. After electrophoresis the proteins are visualized by silver staining. Proteolysis is evident by the appearance of lower molecular weight bands as compared to the negative control.

25 *Inhibition of alpha-2-macroglobulin proteolysis by inhibitors of metalloproteinases*

Known metalloproteinase inhibitors (metal chelators (EDTA, EGTA, AND HgCl₂), peptide metalloproteinase inhibitors (TIMP-1 and TIMP-2), and commercial small molecule 30 MMP inhibitors) may also be used to characterize the proteolytic activity of an albumin fusion protein of the invention. Three synthetic MMP inhibitors that may be used are: MMP inhibitor I, [IC₅₀ = 1.0 μM against MMP-1 and MMP-8; IC₅₀ = 30 μM against MMP-9; IC₅₀ = 150 μM against MMP-3]; MMP-3 (stromelysin-1) inhibitor I [IC₅₀ = 5 μM against MMP-3], and MMP-3 inhibitor II [K_i = 130 nM against MMP-3]; inhibitors available through Calbiochem, catalog # 444250, 444218, and 444225, respectively). Briefly, different 35 concentrations of the small molecule MMP inhibitors are mixed with a purified fusion protein of the invention (50μg/ml) in 22.9 μl of 1x HEPES buffer (50 mM HEPES, pH 7.5, 0.2 M

NaCl, 10 mM CaCl₂, 25 μ M ZnCl₂ and 0.05% Brij-35) and incubated at room temperature (24 °C) for 2-hr, then 7.1 μ l of substrate alpha-2-macroglobulin (0.2 unit/ml) is added and incubated at 37°C for 20-hr. The reactions are stopped by adding 4x sample buffer and boiled immediately for 5 minutes. After SDS-PAGE, the protein bands are visualized by silver stain.

5

Synthetic Fluorogenic Peptide Substrates Cleavage Assay

The substrate specificity for fusion proteins of the invention with demonstrated metalloproteinase activity may be determined using techniques known in the art, such as using synthetic fluorogenic peptide substrates (purchased from BACHEM Bioscience Inc). Test 10 substrates include, M-1985, M-2225, M-2105, M-2110, and M-2255. The first four are MMP substrates and the last one is a substrate of tumor necrosis factor- α (TNF- α) converting enzyme (TACE). These substrates are preferably prepared in 1:1 dimethyl sulfoxide (DMSO) and water. The stock solutions are 50-500 μ M. Fluorescent assays are performed by using a Perkin Elmer LS 50B luminescence spectrometer equipped with a constant temperature water 15 bath. The excitation λ is 328 nm and the emission λ is 393 nm. Briefly, the assay is carried out by incubating 176 μ l 1x HEPES buffer (0.2 M NaCl, 10 mM CaCl₂, 0.05% Brij-35 and 50 mM HEPES, pH 7.5) with 4 μ l of substrate solution (50 μ M) at 25 °C for 15 minutes, and then adding 20 μ l of a purified fusion protein of the invention into the assay cuvett. The final concentration of substrate is 1 μ M. Initial hydrolysis rates are monitored for 30-min.

20

Example 60: Identification and Cloning of VH and VL domains

One method to identify and clone VH and VL domains from cell lines expressing a particular antibody is to perform PCR with VH and VL specific primers on cDNA made from 25 the antibody expressing cell lines. Briefly, RNA is isolated from the cell lines and used as a template for RT-PCR designed to amplify the VH and VL domains of the antibodies expressed by the EBV cell lines. Cells may be lysed in the TRIzol® reagent (Life Technologies, Rockville, MD) and extracted with one fifth volume of chloroform. After addition of chloroform, the solution is allowed to incubate at room temperature for 10 minutes, and the centrifuged at 14,000 rpm for 15 minutes at 4°C in a tabletop centrifuge. The supernatant is collected and RNA is precipitated using an equal volume of isopropanol. 30 Precipitated RNA is pelleted by centrifuging at 14,000 rpm for 15 minutes at 4°C in a tabletop centrifuge. Following centrifugation, the supernatant is discarded and washed with 75%

ethanol. Following washing, the RNA is centrifuged again at 800 rpm for 5 minutes at 4°C. The supernatant is discarded and the pellet allowed to air dry. RNA is then dissolved in DEPC water and heated to 60°C for 10 minutes. Quantities of RNA can be determined using optical density measurements.

5 cDNA may be synthesized, according to methods well-known in the art, from 1.5-2.5 micrograms of RNA using reverse transcriptase and random hexamer primers. cDNA is then used as a template for PCR amplification of VH and VL domains. Primers used to amplify VH and VL genes are shown in Table 3. Typically a PCR reaction makes use of a single 5' primer and a single 3' primer. Sometimes, when the amount of available RNA template is
10 limiting, or for greater efficiency, groups of 5' and/or 3' primers may be used. For example, sometimes all five VH-5' primers and all JH3' primers are used in a single PCR reaction. The PCR reaction is carried out in a 50 microliter volume containing 1X PCR buffer, 2mM of each dNTP, 0.7 units of High Fidelity Taq polymerase, 5' primer mix, 3' primer mix and 7.5 microliters of cDNA. The 5' and 3' primer mix of both VH and VL can be made by pooling
15 together 22 pmole and 28 pmole, respectively, of each of the individual primers. PCR conditions are: 96°C for 5 minutes; followed by 25 cycles of 94°C for 1 minute, 50°C for 1 minute, and 72°C for 1 minute; followed by an extension cycle of 72°C for 10 minutes. After the reaction is completed, sample tubes are stored 4°C.

Table 3: Primer Sequences Used to Amplify VH and VL domains.

	Primer name	SEQ ID NO	Primer Sequence (5'-3')	
VH Primers				
5	Hu VH1-5'	36	CAGGTGCAGCTGGTGCAGTCTGG	
	Hu VH2-5'	37	CAGGTCAACTTAAGGGAGTCTGG	
	Hu VH3-5'	38	GAGGTGCAGCTGGTGGAGTCTGG	
	Hu VH4-5'	39	CAGGTGCAGCTGCAGGAGTCGGG	
	Hu VH5-5'	40	GAGGTGCAGCTGTTGCAGTCTGC	
10	Hu VH6-5'	41	CAGGTACAGCTGCAGCAGTCAGG	
	Hu JH1,2-5'	42	TGAGGAGACGGTGACCAAGGGTGC	
	Hu JH3-5'	43	TGAAGAGACGGTGACCAATTGTCCC	
	Hu JH4,5-5'	44	TGAGGAGACGGTGACCAAGGGTCC	
	Hu JH6-5'	45	TGAGGAGACGGTGACCGTGGTCCC	
15	VL Primers			
	Hu Vkappa1-5'	46	GACATCCAGATGACCCAGTCTCC	
	Hu Vkappa2a-5'	47	GATGTTGTGATGACTCAGTCTCC	
	Hu Vkappa2b-5'	48	GATATTGTGATGACTCAGTCTCC	
20	Hu Vkappa3-5'	49	GAAATTGTGTTGACGCAGTCTCC	
	Hu Vkappa4-5'	50	GACATCGTGATGACCCAGTCTCC	
	Hu Vkappa5-5'	51	GAAACGACACTCACGCAGTCTCC	
	Hu Vkappa6-5'	52	GAAATTGTGCTGACTCAGTCTCC	
	Hu Vlambda1-5'	53	CAGTCTGTGTTGACGCAGCCGCC	
25	Hu Vlambda2-5'	54	CAGTCTGCCCTGACTCAGCCTGC	
	Hu Vlambda3-5'	55	TCCTATGTGCTGACTCAGCCACC	
	Hu Vlambda3b-5'	56	TCTTCTGAGCTGACTCAGGACCC	
	Hu Vlambda4-5'	57	CACGTTATACTGACTCAACCGCC	
	Hu Vlambda5-5'	58	CAGGCTGTGCTCACTCAGCCGTC	
30	Hu Vlambda6-5'	59	AATTTTATGCTGACTCAGCCCCA	
	Hu Jkappa1-3'	60	ACGTTTGATTTCCACCTTGGTCCC	
	Hu Jkappa2-3'	61	ACGTTTGATCTCCAGCTTGGTCCC	
	Hu Jkappa3-3'	62	ACGTTTGATATCCACTTTGGTCCC	
	Hu Jkappa4-3'	63	ACGTTTGATCTCCACCTTGGTCCC	
35	Hu Jkappa5-3'	64	ACGTTTAATCTCCAGTCGTGTC	
	Hu Jlambda1-3'	65	TCAGTCTGTGTTGACGCAGCCGCC	
	Hu Jlambda2-3'	66	CAGTCTGCCCTGACTCAGCCTGC	
	Hu Jlambda3--3'	67	TCCTATGTGCTGACTCAGCCACC	
	Hu Jlambda3b-3'	68	TCTTCTGAGCTGACTCAGGACCC	
40	Hu Jlambda4-3'	69	CACGTTATACTGACTCAACCGCC	
	Hu Jlambda5-3'	70	CAGGCTGTGCTCACTCAGCCGTC	
	Hu Jlambda6-3'	71	AATTTTATGCTGACTCAGCCCCA	

PCR samples are then electrophoresed on a 1.3% agarose gel. DNA bands of the expected sizes (~506 base pairs for VH domains, and 344 base pairs for VL domains) can be cut out of the gel and purified using methods well known in the art. Purified PCR products can be ligated into a PCR cloning vector (TA vector from Invitrogen Inc., Carlsbad, CA).
5 Individual cloned PCR products can be isolated after transfection of *E. coli* and blue/white color selection. Cloned PCR products may then be sequenced using methods commonly known in the art.

The PCR bands containing the VH domain and the VL domains can also be used to create full-length Ig expression vectors. VH and VL domains can be cloned into vectors
10 containing the nucleotide sequences of a heavy (e.g., human IgG1 or human IgG4) or light chain (human kappa or human lambda) constant regions such that a complete heavy or light chain molecule could be expressed from these vectors when transfected into an appropriate host cell. Further, when cloned heavy and light chains are both expressed in one cell line (from either one or two vectors), they can assemble into a complete functional antibody
15 molecule that is secreted into the cell culture medium. Methods using polynucleotides encoding VH and VL antibody domain to generate expression vectors that encode complete antibody molecules are well known within the art.

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It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

25 The entire disclosure of each document cited (including patents, patent applications, patent publications, journal articles, abstracts, laboratory manuals, books, or other disclosures) as well as information available through Identifiers specific to databases such as GenBank, GeneSeq, or the CAS Registry, referred to in this application are herein incorporated by reference in their entirety. The specification and sequence listing of each of
30 the following U.S. applications are herein incorporated by reference in their entirety: Application Nos. 09/091,873 filed June 25, 1998; 60/229,358 filed on April 12, 2000; 60/199,384 filed on April 25, 2000; 60/256,931 filed on December 21, 2000, 09/809,269, filed March 16, 2001; 60/277,980, filed March 23, 2001; 09/236,557, filed January 26, 1999; 09/482,273, filed January 13, 2000; 60/234,925, filed November 1, 2000;
35 09/397,945, filed September 17, 1999; 09/296,622, filed April 23, 1999; 60/092,921, filed July 15, 1998; 09/305,736, filed May 5, 1999; 09/781,417, filed February 13, 2001; 60/152,317, filed September 3, 1999; 09/227,357, filed January 8, 1999; and 60/262,066,

filed January 18, 2001; and International Publication Nos. WO98/39446, filed September 11, 1998; WO 00/61625, filed October 19, 2000; WO/00/77022, filed December 21, 2000; and WO/00/76530, filed December 21, 2000.

**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL**

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 96, line 30.

B. IDENTIFICATION OF DEPOSIT

Further deposits are identified on an additional sheet

Name of depositary institution: American Type Culture Collection

Address of depositary institution (*including postal code and country*)

10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit	Accession Number
11 April 2001	Unassigned

C. ADDITIONAL INDICATIONS (<i>leave blank if not applicable</i>)	This information is continued on an additional sheet <input type="checkbox"/>
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (<i>if the indications are not for all designated States</i>)	

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In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

Continued on additional sheets

E. SEPARATE FURNISHING OF INDICATIONS (*leave blank if not applicable*)

The indications listed below will be submitted to the international Bureau later (*specify the general nature of the indications e.g., "Accession Number of Deposit"*)

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The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

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The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

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The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No.: Unassigned**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

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Address of depositary institution (*including postal code and country*)

10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit 11 April 2001	Accession Number Unassigned
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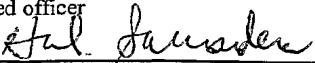
Europe

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ATCC Deposit No.: Unassigned**CANADA**

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NORWAY

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FINLAND

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What is claimed:

1. An albumin fusion protein comprising a Therapeutic protein:X and albumin comprising the amino acid sequence of SEQ ID NO:18.

5

2. An albumin fusion protein comprising a Therapeutic protein:X and a fragment or a variant of the amino acid sequence of SEQ ID NO:18, wherein said fragment or variant has albumin activity.

10 3. The albumin fusion protein of claim 2, wherein said albumin activity is the ability to prolong the shelf life of the Therapeutic protein:X compared to the shelf-life of the Therapeutic protein:X in an unfused state.

15 4. The albumin fusion protein of claim 2, wherein the fragment or variant comprises the amino acid sequence of amino acids 1-387 of SEQ ID NO:18.

5. An albumin fusion protein comprising a fragment or variant of a Therapeutic protein:X, and albumin comprising the amino acid sequence of SEQ ID NO:18, wherein said fragment or variant has a biological activity of the Therapeutic protein:X.

20

6. The albumin fusion protein of any one of claims 1-5, wherein the Therapeutic protein:X, or fragment or variant thereof, is fused to the N-terminus of albumin, or the N-terminus of the fragment or variant of albumin.

25

7. The albumin fusion protein of any one of claims 1-5, wherein the Therapeutic protein:X, or fragment or variant thereof, is fused to the C-terminus of albumin, or the C-terminus of the fragment or variant of albumin.

8. The albumin fusion protein of any one of claims 1-5, wherein the

Therapeutic protein:X, or fragment or variant thereof, is fused to the N- terminus and C- terminus of albumin, or the N-terminus and the C-terminus of the fragment or variant of albumin.

5 9. The albumin fusion protein of any one of claims 1-5, which comprises a first Therapeutic protein:X, or fragment or variant thereof, and a second Therapeutic protein:X, or fragment or variant thereof, wherein said first Therapeutic protein:X, or fragment or variant thereof, is different from said second Therapeutic protein:X, or fragment or variant thereof.

10

10. The albumin fusion protein of any one of claims 1-8, wherein the Therapeutic protein:X, or fragment or variant thereof, is separated from the albumin or the fragment or variant of albumin by a linker.

15

11. The albumin fusion protein of any one of claims 1-8, wherein the albumin fusion protein has the following formula:

R1-L-R2; R2-L-R1; or R1-L-R2-L-R1,

20 wherein R1 is Therapeutic protein:X, or fragment or variant thereof, L is a peptide linker, and R2 is albumin comprising the amino acid sequence of SEQ ID NO:18 or fragment or variant of albumin.

12. The albumin fusion protein of any one of claims 1-11, wherein the shelf-life of the albumin fusion protein is greater than the shelf-life of the Therapeutic protein:X in an unfused state.

25

13. The albumin fusion protein of any one of claims 1-11, wherein the in vitro biological activity of the Therapeutic protein:X, or fragment or variant thereof, fused to albumin, or fragment or variant thereof, is greater than the in vitro biological activity of the Therapeutic protein:X, or a fragment or variant thereof, in an unfused state.

14. The albumin fusion protein of any one of claims 1-11, wherein the in vivo biological activity of the Therapeutic protein:X, or fragment or variant thereof, fused to albumin, or fragment or variant thereof, is greater than the in vivo biological activity of the
5 Therapeutic protein:X , or a fragment or variant thereof, in an unfused state.

15. An albumin fusion protein comprising a Therapeutic protein:X, or fragment or variant thereof, inserted into an albumin comprising the amino acid sequence of SEQ ID NO:18 or fragment or variant thereof.

10

16. An albumin fusion protein comprising a Therapeutic protein:X, or fragment or variant thereof, inserted into an albumin comprising an amino acid sequence selected from the group consisting of:

- (a) amino acids 54 to 61 of SEQ ID NO:18;
- (b) amino acids 76 to 89 of SEQ ID NO:18;
- (c) amino acids 92 to 100 of SEQ ID NO:18;
- (d) amino acids 170 to 176 of SEQ ID NO:18;
- (e) amino acids 247 to 252 of SEQ ID NO:18;
- (f) amino acids 266 to 277 of SEQ ID NO:18;
- 15 (g) amino acids 280 to 288 of SEQ ID NO:18;
- (h) amino acids 362 to 368 of SEQ ID NO:18;
- (i) amino acids 439 to 447 of SEQ ID NO:18;
- (j) amino acids 462 to 475 of SEQ ID NO:18;
- 20 (k) amino acids 478 to 486 of SEQ ID NO:18; and
- (l) amino acids 560 to 566 of SEQ ID NO:18.

25

17. The albumin fusion protein of claims 15 or 16, wherein said albumin fusion protein comprises a portion of albumin sufficient to prolong the shelf-life of the Therapeutic protein:X, or fragment or variant thereof, as compared to the shelf-life of the Therapeutic

protein:X, or a fragment or variant thereof, in an unfused state.

18. The albumin fusion protein of claims 15 or 16, wherein said albumin fusion protein comprises a portion of albumin sufficient to prolong the in vitro biological activity of the Therapeutic protein:X, or fragment or variant thereof, fused to albumin as compared to the in vitro biological activity of the Therapeutic protein:X, or a fragment or variant thereof, in an unfused state.

19. The albumin fusion protein of claims 15 or 16 wherein said albumin fusion protein comprises a portion of albumin sufficient to prolong the in vivo biological activity of the Therapeutic protein:X, or fragment or variant thereof, fused to albumin compared to the in vivo biological activity of the Therapeutic protein:X, or a fragment or variant thereof, in an unfused state.

15 20. The albumin fusion protein of any one of claims 1-19, which is non-glycosylated.

21. The albumin fusion protein of any one of claims 1-19, which is expressed in yeast.

20 22. The albumin fusion protein of claim 21, wherein the yeast is glycosylation deficient.

25 23. The albumin fusion protein of claim 21 wherein the yeast is glycosylation and protease deficient.

24. The albumin fusion protein of any one of claims 1-19, which is expressed by a mammalian cell.

25. The albumin fusion protein of any one of claims 1-19, wherein the albumin fusion protein is expressed by a mammalian cell in culture.

5 26. The albumin fusion protein of any one of claims 1-19, wherein the albumin fusion protein further comprises a secretion leader sequence.

27. A composition comprising the albumin fusion protein of any one of claims 1-26 and a pharmaceutically acceptable carrier.

10 28. A kit comprising the composition of claim 27.

29. A method of treating a disease or disorder in a patient, comprising the step of administering the albumin fusion protein of any one of claims 1-26.

15 30. The method of claim 29, wherein the disease or disorder comprises indication:Y.

20 31. A method of treating a patient with a disease or disorder that is modulated by Therapeutic protein:X, or fragment or variant thereof, comprising the step of administering an effective amount of the albumin fusion protein of any one of claims 1-26.

32. The method of claim 31, wherein the disease or disorder is indication:Y.

25 33. A method of extending the shelf life of Therapeutic protein:X comprising the step of fusing the Therapeutic protein:X, or fragment or variant thereof, to albumin or a fragment or variant thereof, sufficient to extend the shelf-life of the Therapeutic protein:X, or fragment or variant thereof, compared to the shelf-life of the Therapeutic protein:X, or a fragment or variant thereof, in an unfused state.

34. A nucleic acid molecule comprising a polynucleotide sequence encoding the albumin fusion protein of any one of claims 1-26.

35. A vector comprising the nucleic acid molecule of claim 34.

5

36. A host cell comprising the nucleic acid molecule of claim 35.

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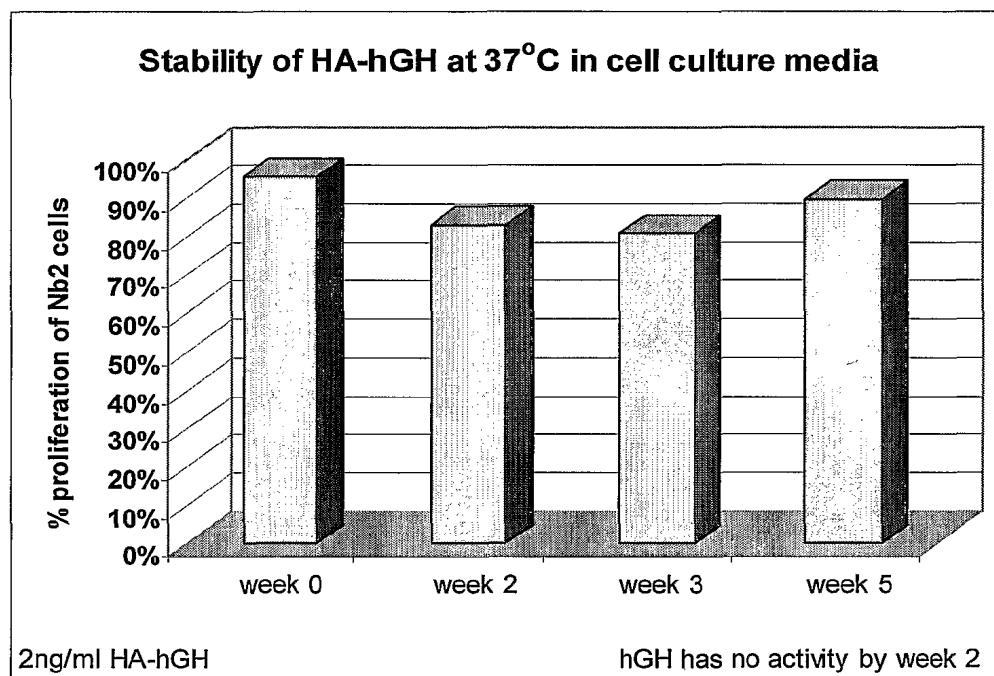
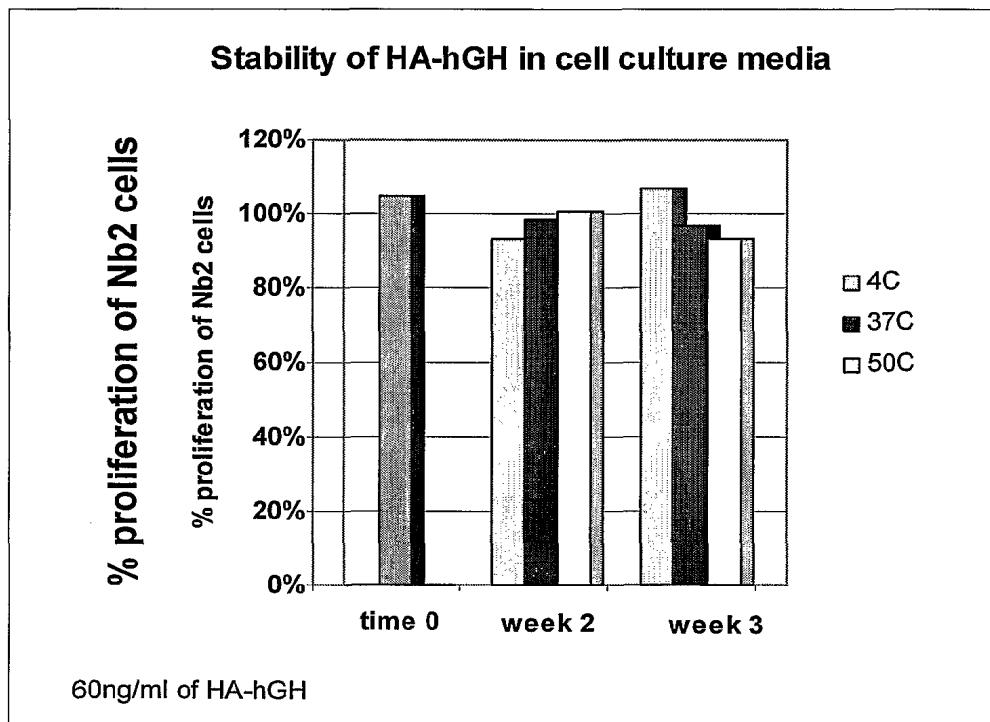
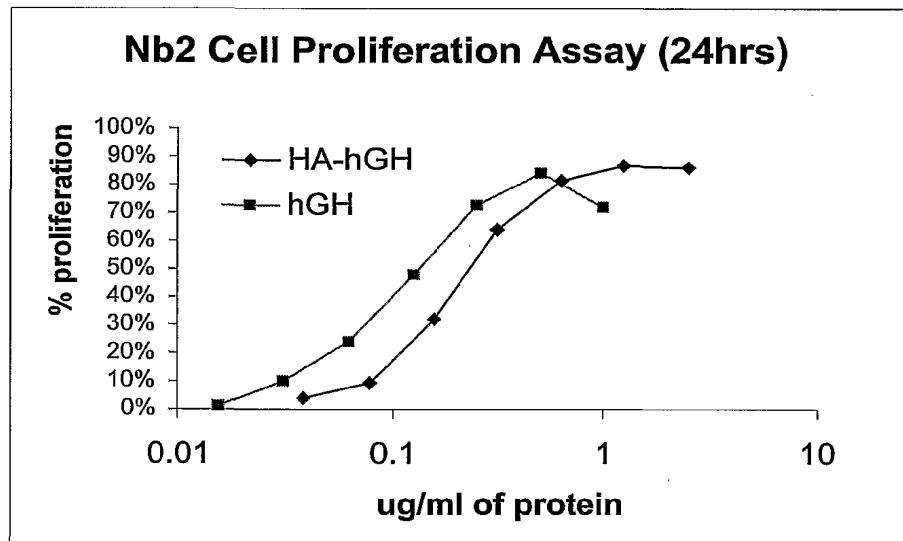
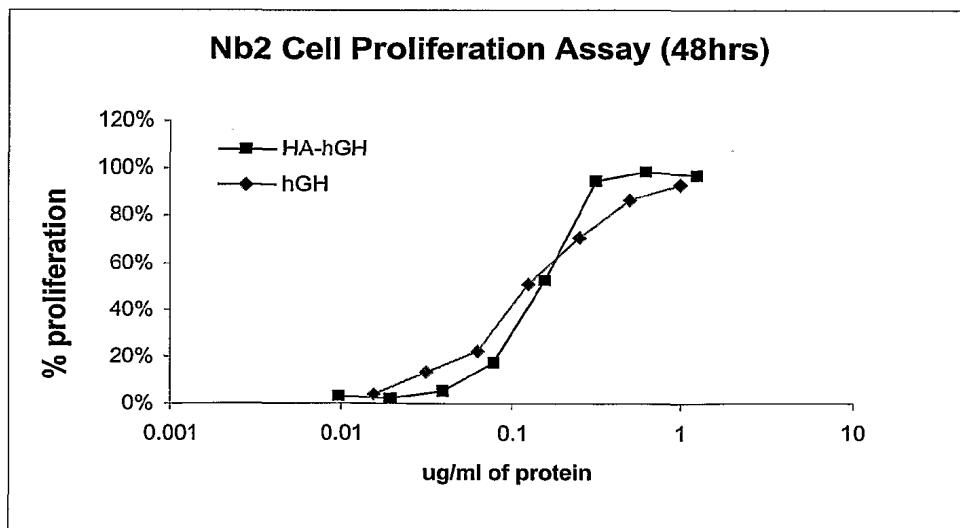


Figure 1

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**Figure 2**

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**Figure 3A****Figure 3B**

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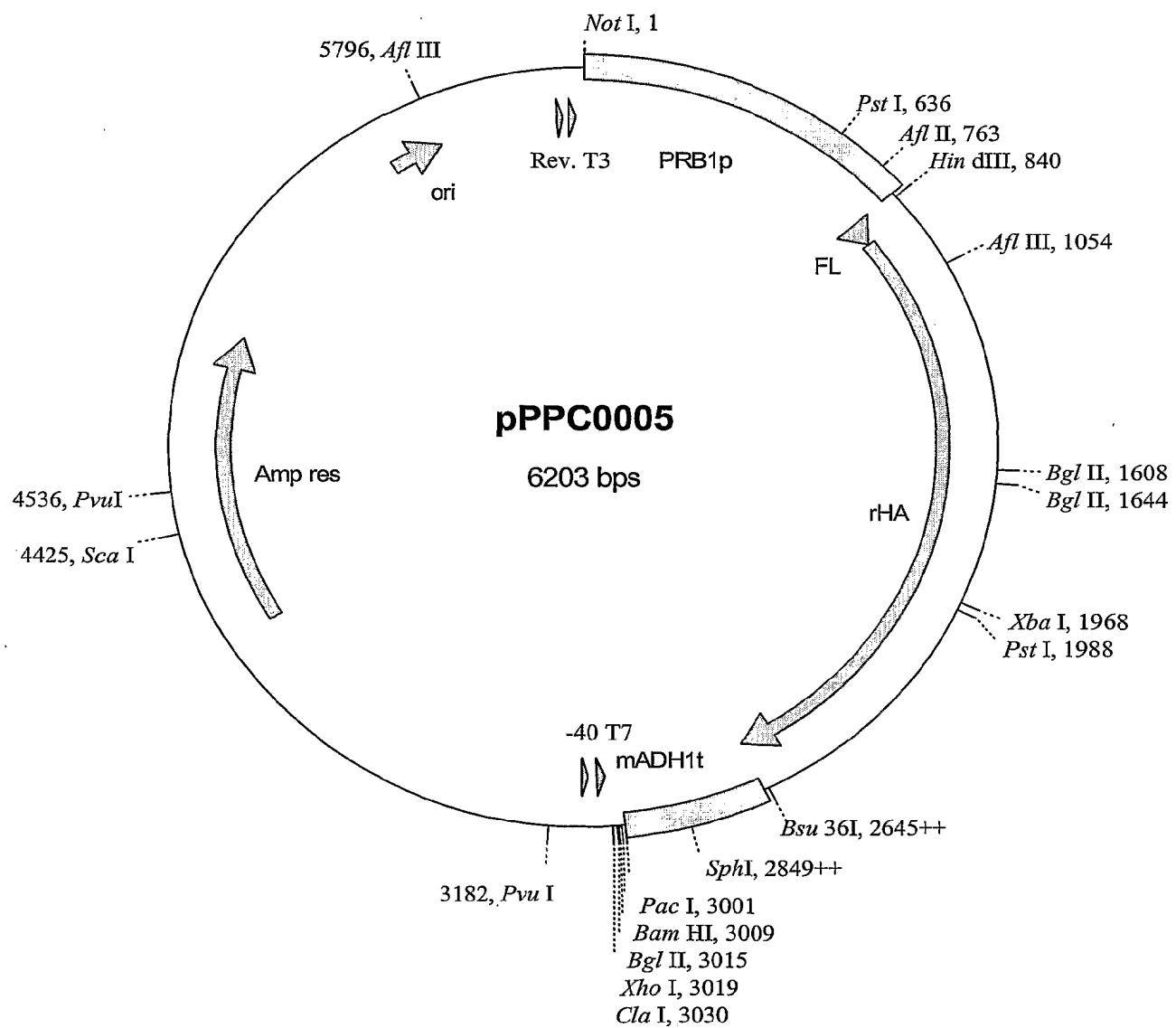
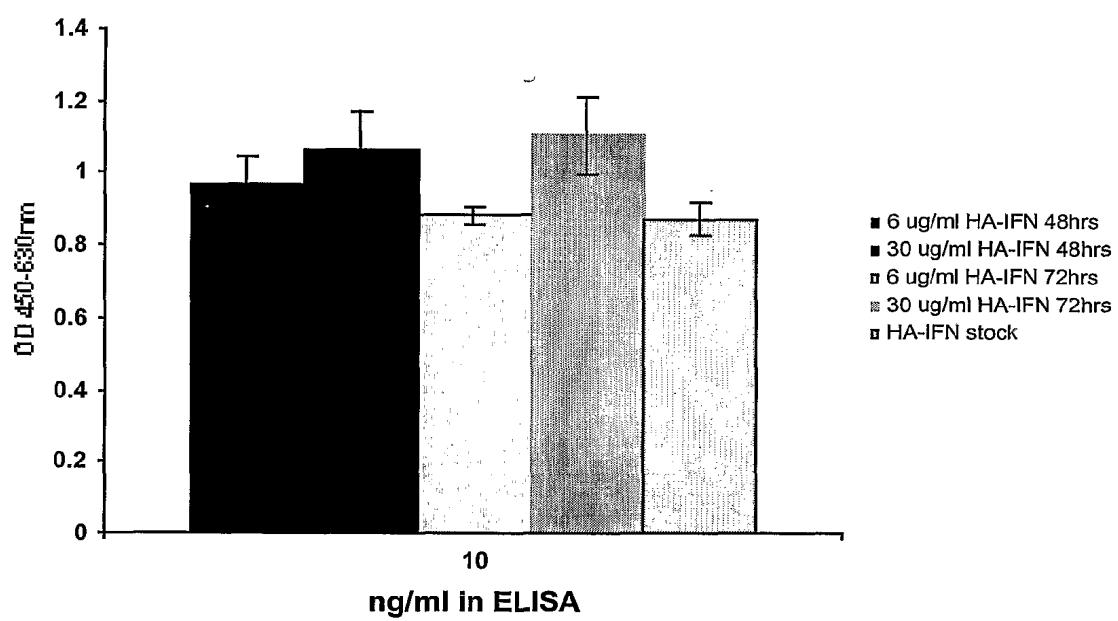


Figure 4

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**Figure 5**

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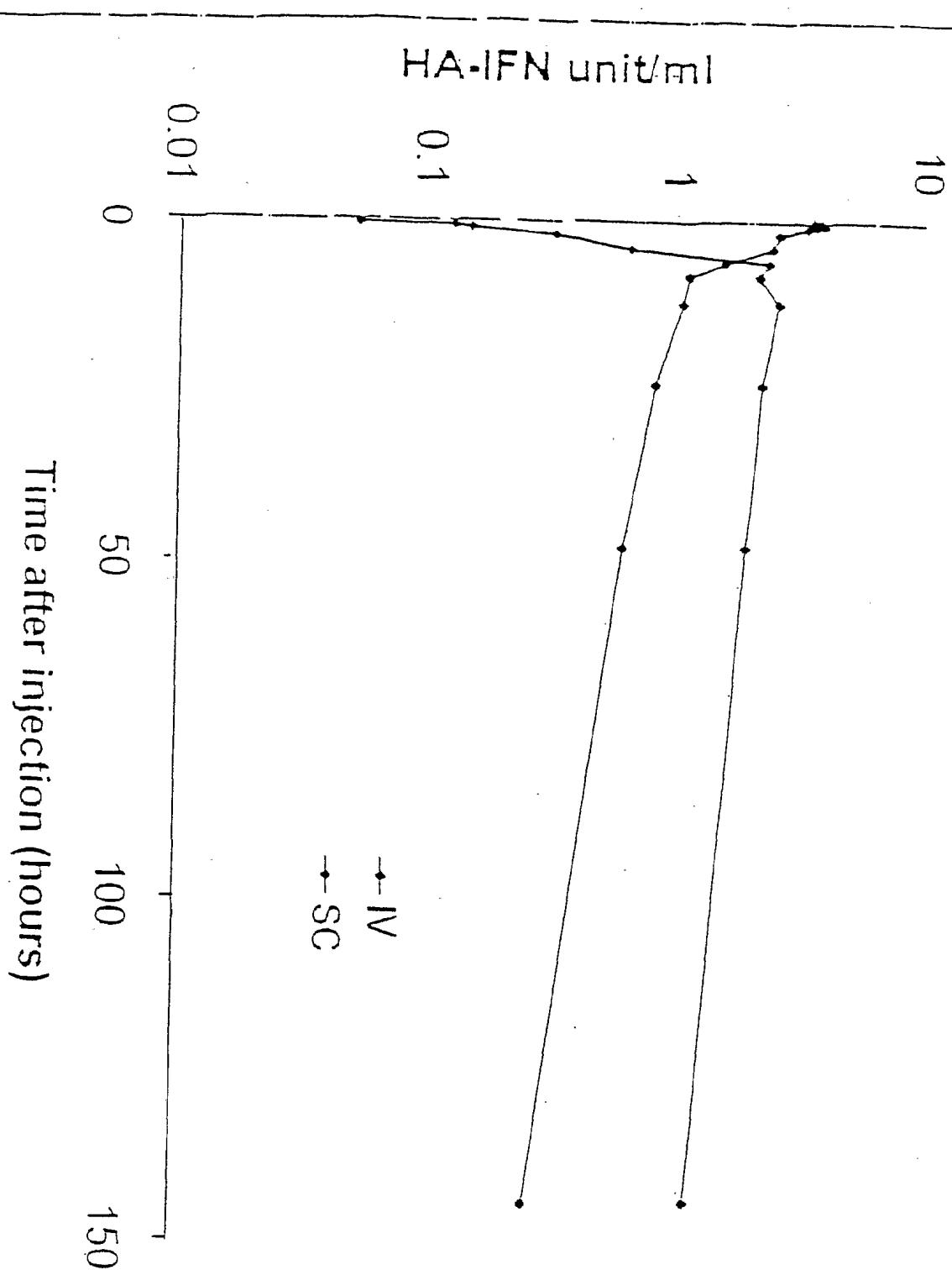


Figure 6

7/20

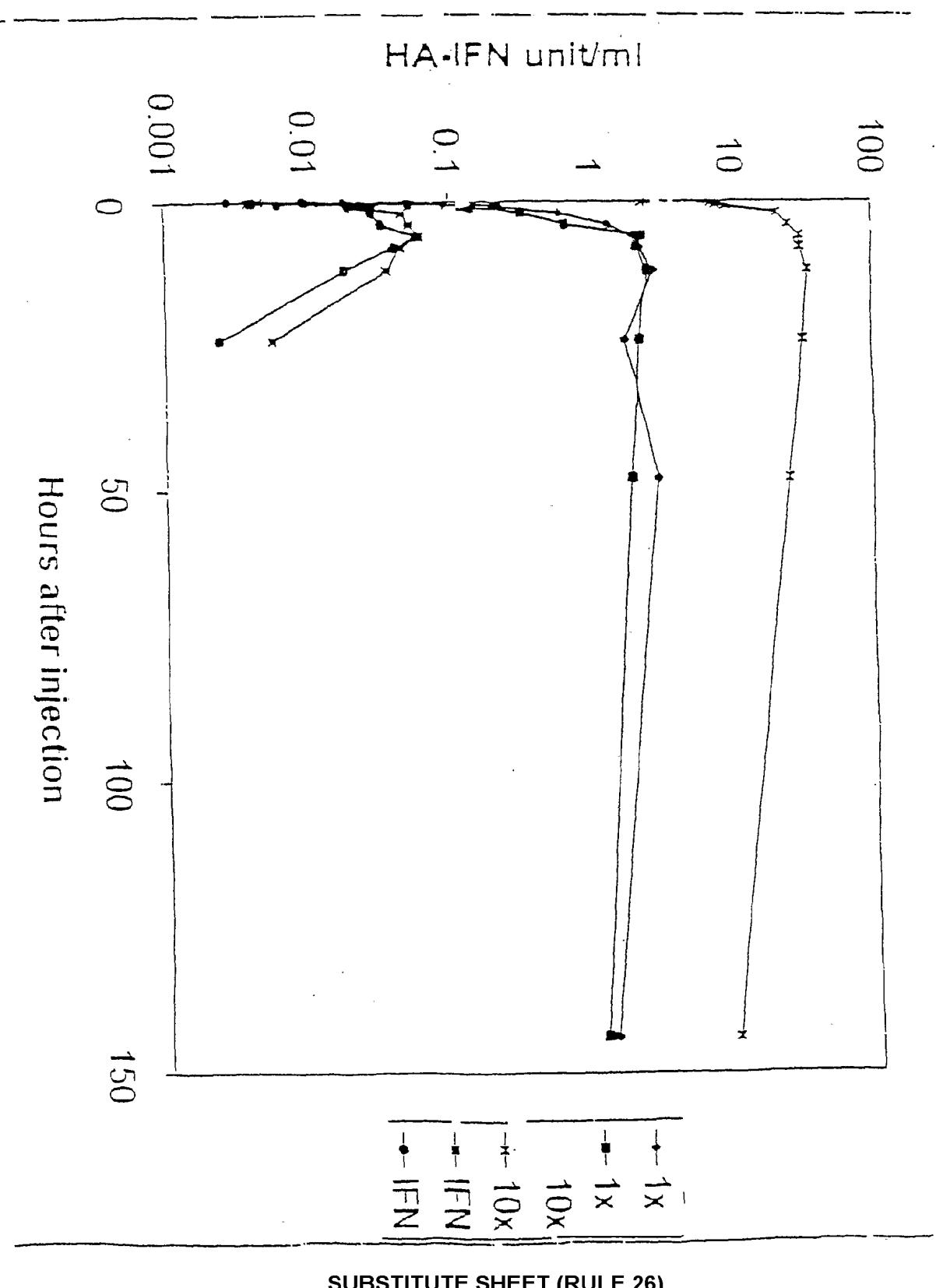
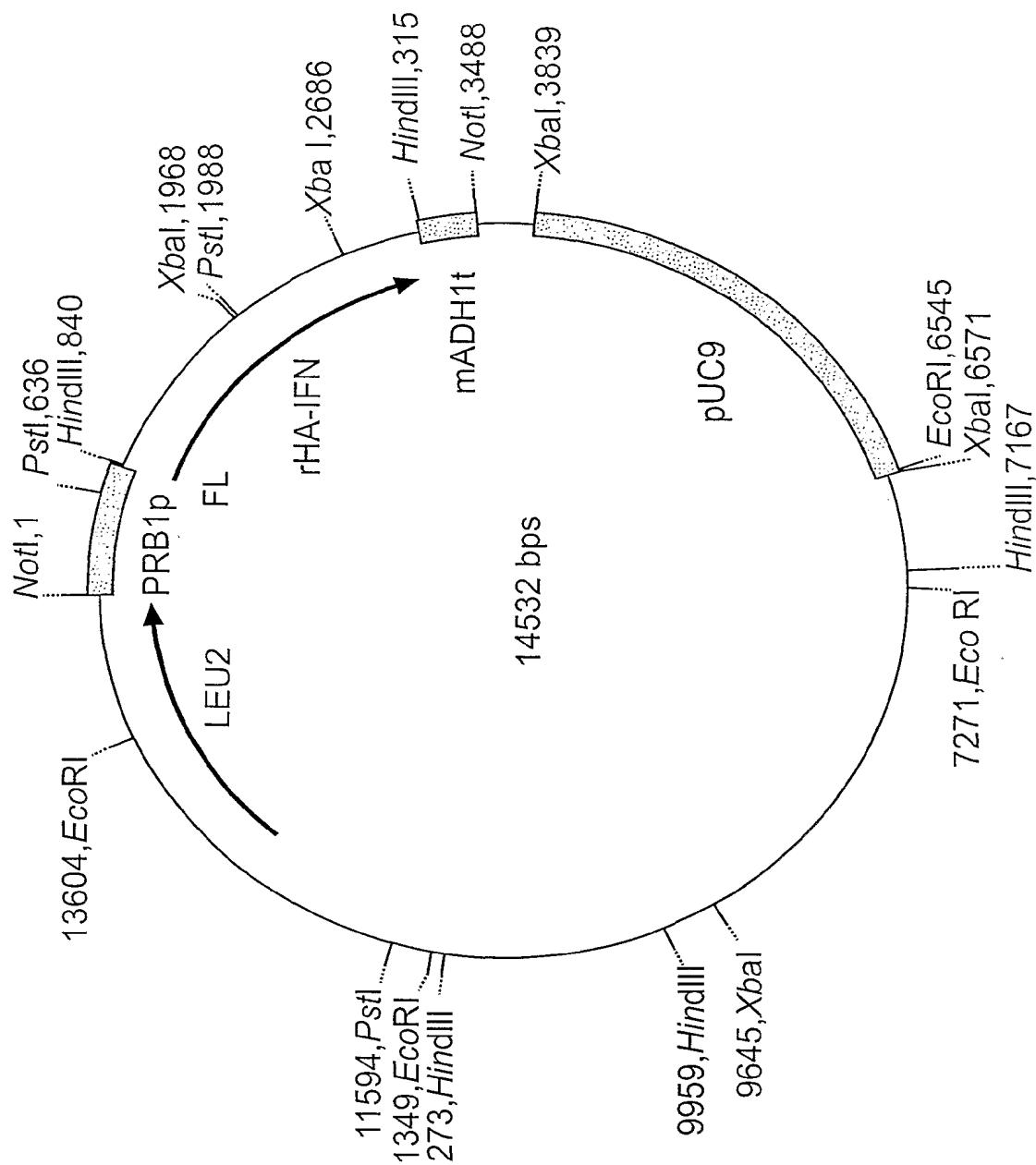


Figure 7

**FIG. 8**

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Localisation of ‘Loops’ based on the HA Crystal Structure which could be used for Mutation/Insertion

I	II	III
51 KTCV <u>ADESAE</u> NCDKSLHTLF GDKL <u>CTVATL</u> RETY <u>GE</u> MADC CA <u>KO</u> EPERNE		
HHHHH HHHHH HHHHH	HHHH H	HHHH

201 ASLQKFGERA FKA
V
HGGDL

VI VII
251 LECADDRADL AKYICENODS ISSKILKECCE KPLLEKSHCI AEVENDEMPA
.....

301 DLPSLAADFV ESKDVCKNYA EAKDVFLGMF LYEYARRHPD YSVVLLLRILA
 HHHH HHHHHH HHHHHHHH HHHHHH HHHHHHHH HHHHHHHH

351 KTYETTLEKC AAADPHECY AKVFDEFKPL VEEPQNLIKQ NCELFEQLGE

401 YKFQNALLVR YTKKVPQVST PTLVEVSRNL GKVGSKCCKH PEAKRMPCAE

X XI
451 DYLSVVLNQL **CVLHEKTPVS DRVTKCCTES LVNRRP** PCFSA LEVDETYVPK

501 EFNAETFTFH ADICTLSEKE RQIKKKQTALV ELVKHKPKAT KEQLKAVMDD

XII

AAAAAA AAAAAA AAAAAA A

Loop	Loop
I Val54-Asn61	VII Glu280-His288
II Thr76-Asp89	VIII Ala362-Glu368
III Ala92-Glu100	IX Lys439-Pro447
IV Gln170-Ala176	X Val462-Lys475
V His247-Glu252	XI Thr478-Pro486
VI Glu266-Glu277	XII Lys560-Thr566

Figure 9

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Examples of Modifications to Loop IV**a. Randomisation of Loop IV.**

IV

151 APELLFFAKR YKAAFTECCQ AADKAACLLP KLDELRDEGK ASSAKQRLKC
 HHHHHHHHHHHH HHHHHHHHHHHH HHHHH HHHHHHHHHHHH HHHHHHHHHHHH

IV

151 APELLFFAKR YKAAFTECCX XXXXXXCLLP KLDELRDEGK ASSAKQRLKC
 HHHHHHHHHHHH HHHHHHHHHHHH HHHHH HHHHHHHHHHHH HHHHHHHHHHHH

X represents the mutation of the natural amino acid to any other amino acid. One, more or all of the amino acids can be changed in this manner. This figure indicates all the residues have been changed.

b. Insertion (or replacement) of Randomised sequence into Loop IV.

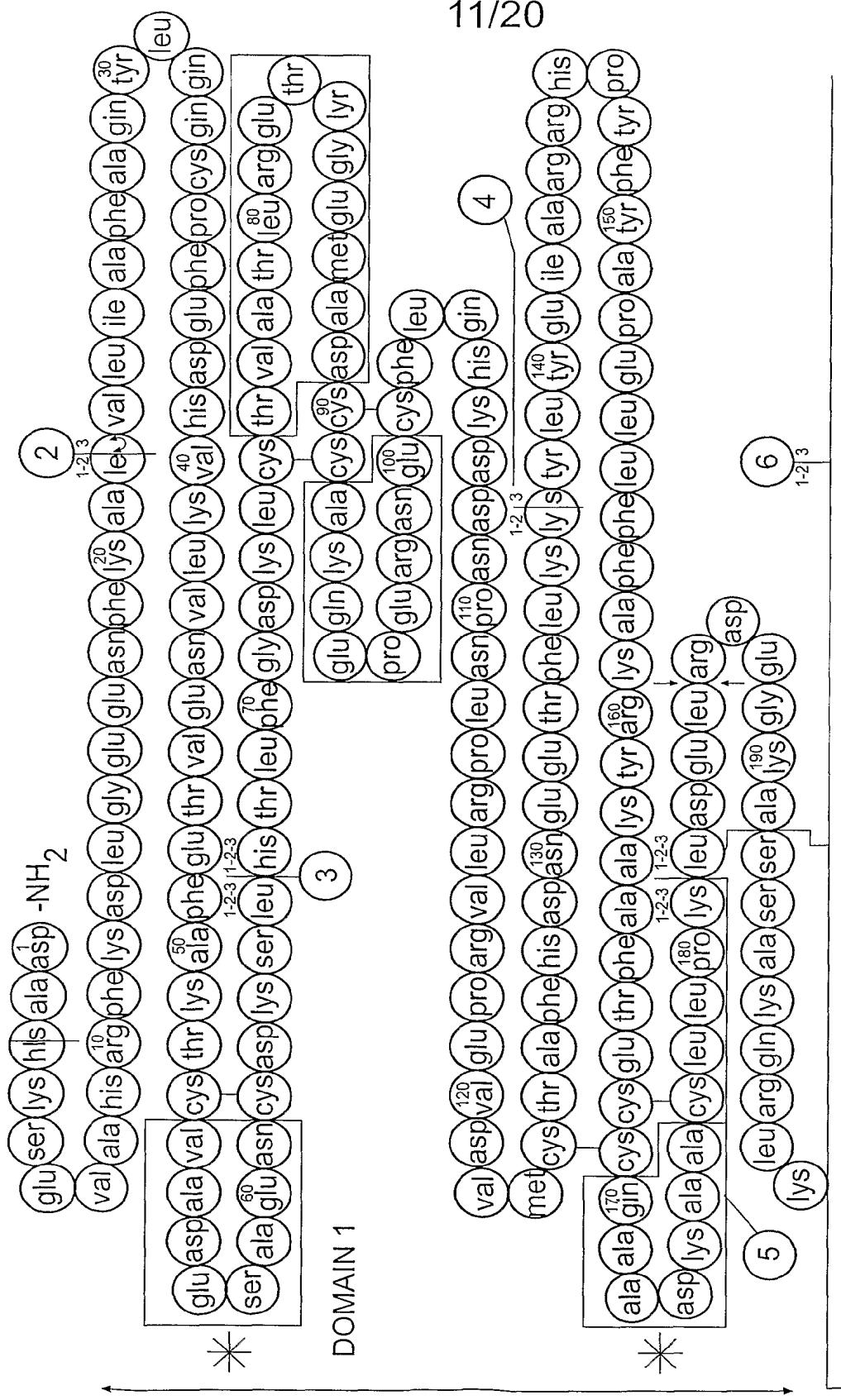
(X)_n
 ↓
 IV

151 APELLFFAKR YKAAFTECCQ AADKAACLLP KLDELRDEGK ASSAKQRLKC
 HHHHHHHHHHHH HHHHHHHHHHHH HHHHH HHHHHHHHHHHH HHHHHHHHHHHH

The insertion can be at any point on the loop and the length a length where n would typically be 6, 8, 12, 20 or 25.

Figure 10

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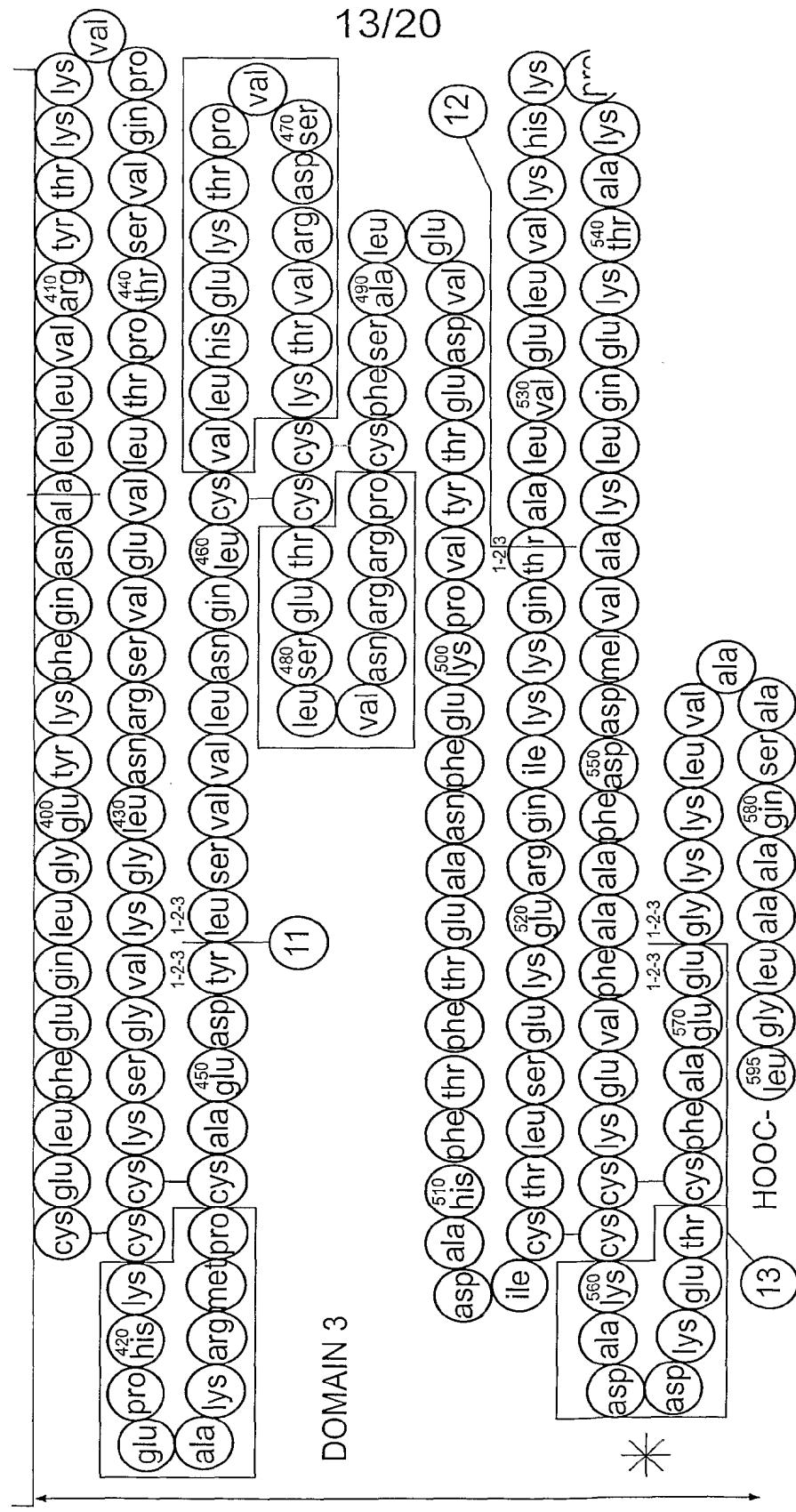
SUBSTITUTE SHEET (RULE 26)

TO FIG. 11B

FIG. 11A

FROM FIG. 11B

FROM FIG. 11B



SUBSTITUTE SHEET (RULE 26)

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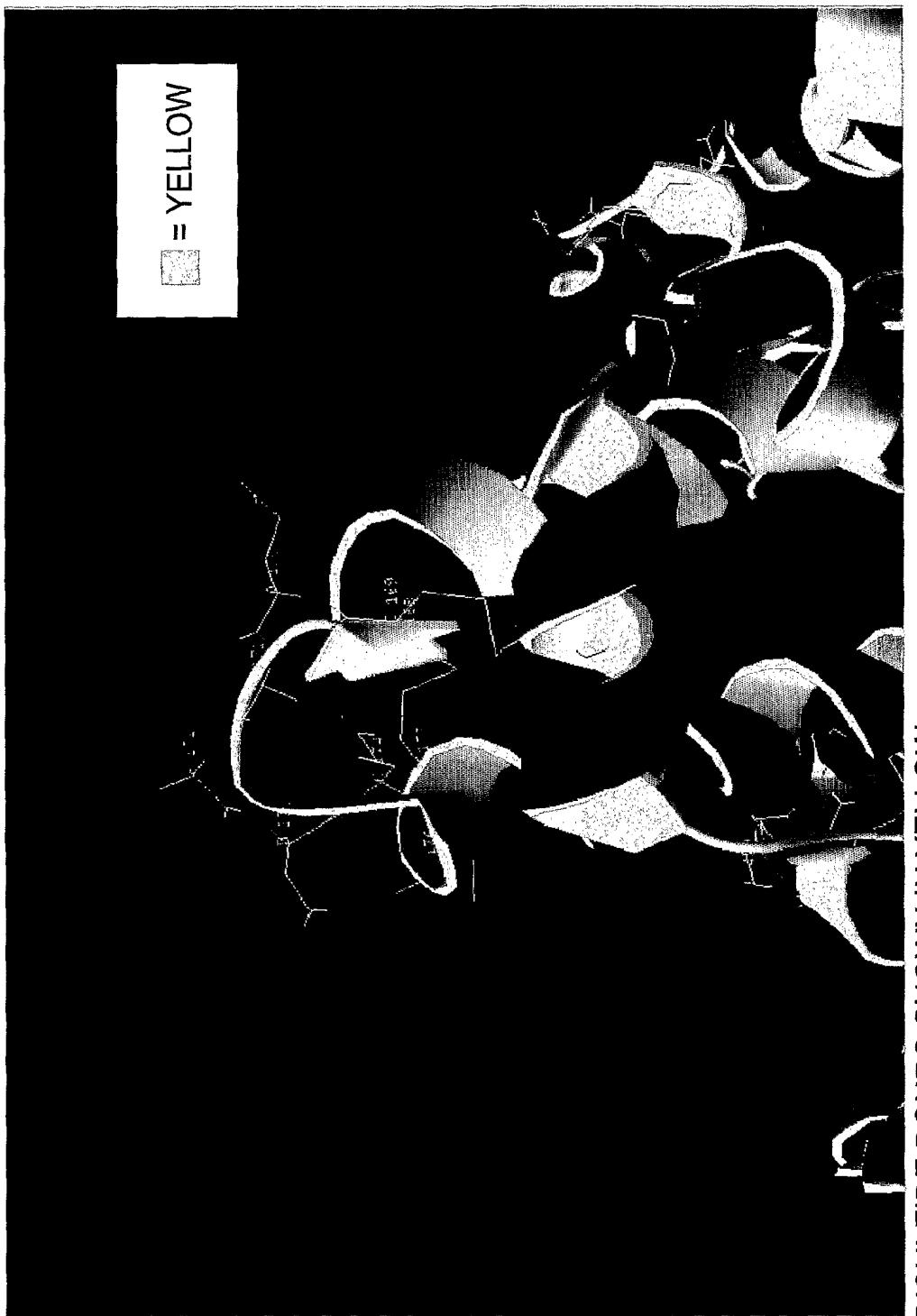


FIG. 12:
LOOP IV GLU170-A176

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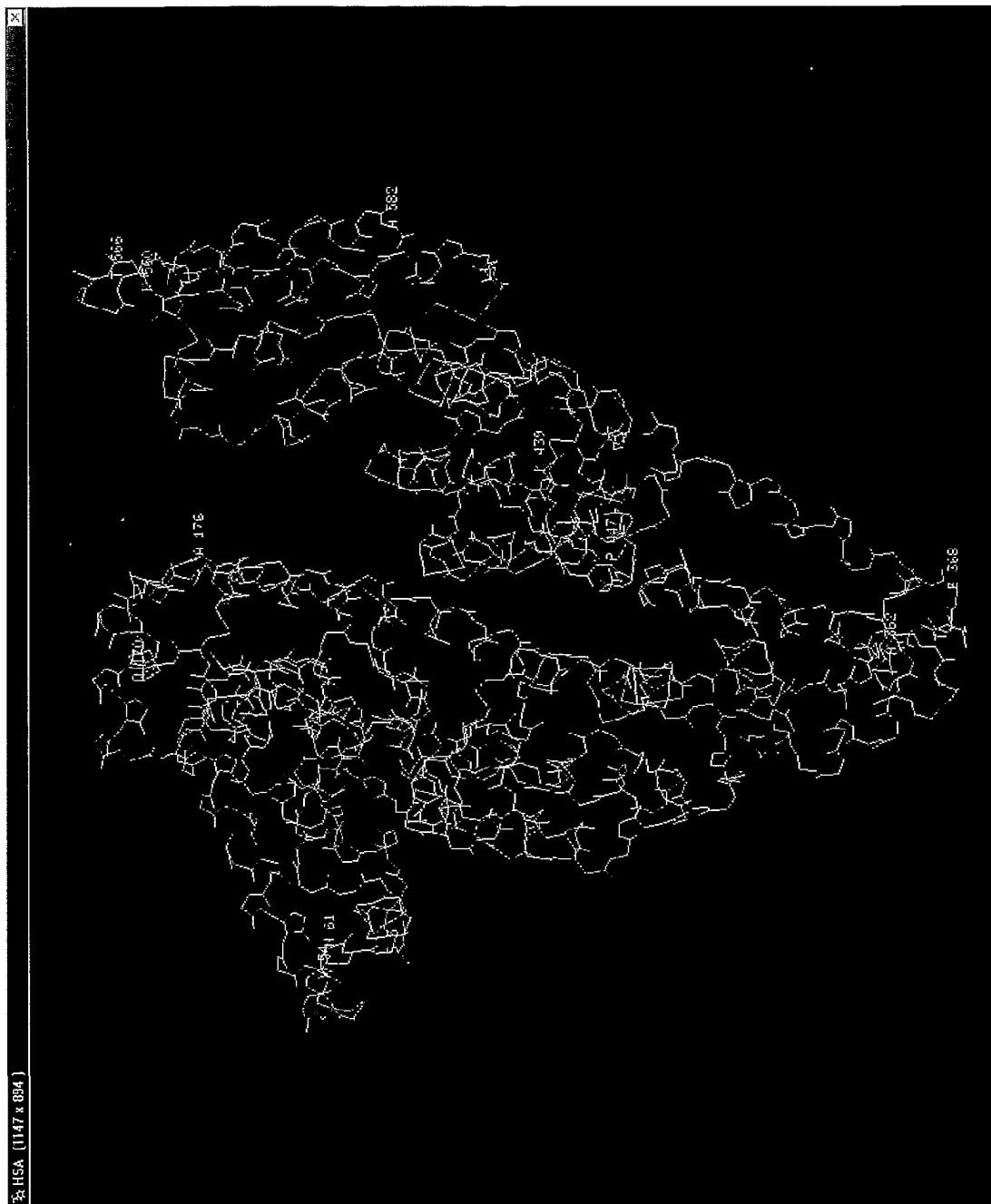


FIG. 13
TERTIARY STRUCTURE OF HSA

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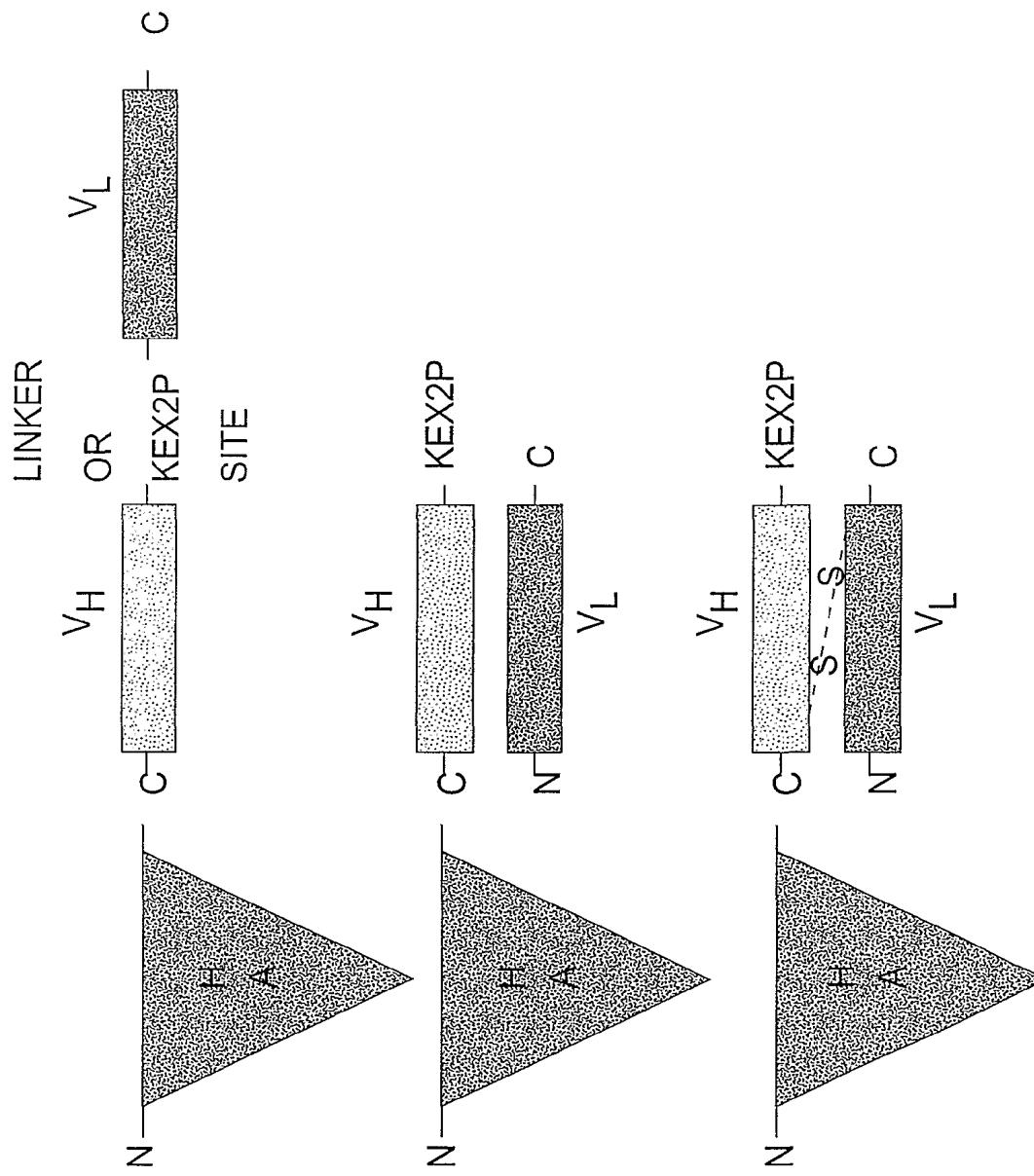


FIG. 14

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1 GAT GCA CAC AAG AGT GAG GTP GCT CAT CGG TTT AAA GAT TTG GGA GAA AAT TTC AAA 60
 1 D A H K S E V A H R F K D L G E E N F K 20

61 GCC TTG GTG ATT GCC TTT GCT CAG TAT CTT CAG CAG TGT CCA TTT GAA GAT CAT GTA 120
 21 A L V L I A F A Q Y L Q Q C P F E D H V 40

121 AAA TTA GTG AAT GAA GTA ACT GAA TTT GCA AAA ACA TGT GTT GCT GAT GAG TCA GCT GAA 180
 41 K L V N E V T E F A K T C V A D E S A E 60

181 AAT TGT GAC AAA TCA CTT CAT ACC CTT TTT GGA GAC AAA TTA TGC ACA GTT GCA ACT CTT 240
 61 N C D K S L H T L F G D K L C T V A T L 80

241 CGT GAA ACC TAT GGT GAA ATG GCT GAC TGC TGT GCA AAA CAA GAA CCT GAG AGA AAT GAA 300
 81 R E T Y G E M A D C C A K Q E P E R N E 100

301 TGC TTC TTG CAA CAC AAA GAT GAC AAC CCA AAC CTC CCC CGA TTG GTG AGA CCA GAG GTT 360
 101 C F L Q H K D D N P N L P R L V R P E V 120

361 GAT GTG ATG TGC ACT GCT TTT CAT GAC AAT GAA GAG ACA TTT TTG AAA AAA TAC TTA TAT 420
 121 D V M C T A F H D N E E T F L K K Y L Y 140

421 GAA ATT GCC AGA AGA CAT CCT TAC TTT TAT GCC CCG GAA CTC CTT TTC TTT GCT AAA AGG 480
 141 E I A R R H P Y F V A P E L L F A K R 160

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481	TAT	AAA	GCT	TTT	ACA	GAA	TGT	TGC	CAA	GCT	GAT	AAA	GCT	GCC	TGC	CTG	TTG	CCA	540		
161	Y	K	A	A	F	T	E	C	C	Q	A	A	D	K	A	C	L	L	P	180	
541	AAG	CTC	GAT	GAA	CTT	CGG	GAT	GAA	GGG	AAG	GCT	TGC	TCT	GCC	AAA	CAG	AGA	CTC	AAA	TGT	600
181	K	L	D	E	L	R	D	E	G	K	A	S	S	A	K	Q	R	L	K	C	200
601	GCC	AGT	CTC	CAA	AAA	TTT	GGA	GAA	AGA	GCT	TTC	AAA	GCA	TGG	GCA	GTG	GCT	CGC	CTG	AGC	660
201	A	S	L	Q	K	F	G	E	R	A	F	K	A	W	A	V	A	R	L	S	220
661	CAG	AGA	TTT	CCC	AAA	GCT	GAG	TTT	GCA	GAA	GTT	TCC	AAG	TAA	GTG	ACA	GAT	CTT	ACC	AAA	720
221	Q	R	F	P	K	A	E	F	A	E	V	S	K	L	V	T	D	L	T	K	240
721	GTC	CAC	ACG	GAA	TGC	TGC	CAT	GGA	GAT	CTG	CTT	GAA	TGT	GCT	GAT	GAC	AGG	GCG	GAC	CTT	780
241	V	H	T	E	C	C	H	G	D	L	L	E	C	A	D	D	R	A	D	L	260
781	GCC	AAG	TAT	ATC	TGT	GAA	AAT	CAG	GAT	TCG	ATC	TCC	AGT	AAA	CTG	AAG	GAA	TGC	TGT	GAA	840
261	A	K	Y	I	C	E	N	Q	D	S	I	S	K	L	K	E	C	C	E	280	
841	AAA	CCT	CTG	TTG	GAA	AAA	TCC	CAC	TCG	ATT	GCC	GAA	GAT	GAG	ATG	CCT	GCT	900			
281	K	P	L	L	E	K	S	H	C	I	A	E	V	E	N	D	E	M	P	A	300
901	GAC	TTG	CCT	TCA	TTA	GCT	GCT	GAT	TTT	GAA	AGT	AAG	GAT	GTT	TGC	AAA	AAC	TAT	GCT	960	
301	D	L	P	S	L	A	A	D	F	V	E	S	K	D	V	C	K	N	Y	A	320

Figure 15B

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961	GAG	GCA	AAG	GAT	GTC	TTC	CTG	GCG	ATG	TTG	TAT	GAA	TAT	GCA	AGA	AGG	CAT	CCT	GAT	1020	
321	E	A	K	D	V	F	L	G	M	F	L	Y	E	A	R	R	R	H	P	D	340
1081	TAC	TCT	GTC	GTG	CTG	CTG	AGA	CCT	GCC	AAG	ACA	TAT	GAA	ACC	ACT	CTA	GAG	AAG	TGC	1080	
341	Y	S	V	V	L	L	R	L	A	K	T	Y	E	T	T	L	E	K	P	C	360
1141	GTG	GAA	GAG	CCT	CAG	AAT	TTC	ATC	AAA	CAA	AAC	TGT	GAG	CTT	TTT	GAG	CAG	CTT	GGA	GAG	1140
381	V	E	E	P	Q	N	L	I	K	Q	N	C	E	L	F	E	Q	L	G	E	380
1201	TAC	AAA	TTC	CAG	AAT	GCG	CTA	TTA	GTT	CGT	TAC	ACC	AAG	AAA	GTA	CCC	CAA	GTG	TCA	ACT	1200
401	Y	K	F	Q	N	A	L	L	V	R	Y	T	K	K	V	P	Q	V	S	T	420
1261	CCA	ACT	CTT	GTA	GAG	GTC	TCA	AGA	AAC	CTA	GGAA	AAA	GTG	GGC	AGC	AAA	TGT	TGT	AAA	CAT	1320
421	P	T	L	V	E	V	S	R	N	L	G	K	V	G	S	K	C	C	K	H	440
1321	CCT	GAA	GCA	AAA	AGA	ATG	CCC	TGT	GCA	GAA	GAC	TAT	CTA	TCC	GTG	GTG	AAC	CAG	TTA	1380	
441	P	E	A	K	R	M	P	C	A	E	D	Y	L	S	V	V	L	N	Q	L	460
1381	TGT	GTG	TTG	CAT	GAG	AAA	ACG	CCA	GTA	AGT	GAC	AGA	GTC	ACA	AAA	TGC	TGC	ACA	GAG	TCC	1440
461	C	V	L	H	E	K	T	P	V	S	D	R	V	T	K	C	C	T	E	S	480

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1441	TG	TG	AAC	AGG	CGA	CCA	TGC	TTT	TCA	GCT	CTG	GAA	GTC	GAT	GAA	ACA	TAC	GTT	CCC	AAA	1500	
481	L	V	N	R	R	P	C	F	S	A	L	E	V	D	E	T	Y	V	P	K	500	
1501	GAG	TTT	AAT	GCT	GAA	ACA	TTC	ACC	TTC	CAT	GCA	GAT	ATA	TGC	ACA	CCT	TCT	GAG	AAG	GAG	1560	
501	E	F	N	A	E	T	F	T	F	H	A	D	I	C	T	L	S	E	K	E	520	
1561	AGA	CAA	ATC	AAG	AAA	CAA	ACT	GCA	CTT	GAG	CTT	GAG	AAA	CAC	AAG	CCC	AAG	GCA	ACA	1620		
521	R	Q	I	K	K	Q	T	A	L	V	E	L	V	K	H	K	P	K	A	T	540	
1621	AAA	GAG	CAA	CTG	AAA	GCT	GTT	ATG	GAT	GAT	TTC	GCA	GCT	TTT	GTA	GAG	AAG	TGC	TGC	AAG	1680	
541	K	E	Q	L	K	A	V	M	D	D	F	A	A	F	V	E	K	C	C	K	560	
1681	GCT	GAC	GAT	AAG	GAG	ACC	TGC	TTT	GCC	GAG	GAG	GGT	AAA	AAA	AAA	CTT	GTT	GCT	GCA	AGT	CAA	1740
561	A	D	K	E	T	C	F	A	E	E	G	K	K	L	V	A	A	S	Q	580		
1741	GCT	GCC	TTA	GGC	TTA	TAA	CAT	CTA	CAT	TTA	AAA	GCA	TCT	CAG	1782							
581	A	A	L	G	L	*															585	

Figure 15D

SEQUENCE LISTING

<110> Human Genome Sciences, Inc.

<120> Albumin Fusion Proteins

<130> PF545PCT

<140> Unassigned

<141> 2001-04-12

<150> 60/229,358

<151> 2000-04-12

<150> 60/256,931

<151> 2000-12-21

<150> 60/199,384

<151> 2000-04-25

<160> 79

<170> PatentIn Ver. 2.1

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<212> DNA

<213> Artificial Sequence

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<400> 1

cccaagaatt cccttatcca ggc

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<210> 2

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33

<210> 3

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<213> Artificial Sequence

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with non-cohesive ends.

<400> 3
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16

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<400> 4
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17

<210> 5
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17

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<400> 6
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18

<210> 7
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<212> PRT
<213> Artificial Sequence

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<223> invertase leader sequence

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<222> 20)..(24)
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<400> 7
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1 5 10 15

Ile Ser Ala Asp Ala His Lys Ser
20

<210> 8
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fragments with non-cohesive ends.

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<210> 12
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<210> 14
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<212> DNA
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aattgttggg aaagatccac caccggcgga tccaccggca cctaagcc 48

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<213> Artificial Sequence

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<400> 15

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ac 62

<210> 16
<211> 63
<212> DNA
<213> Artificial Sequence

<220>
<221> misc_structure
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fragments with non-cohesive ends.

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aattgttggg aaggatccac cgccaccaga tccgcccggca ccagatccac caccgcctaa 60
gcc 63

<210> 17
<211> 1782
<212> DNA
<213> Homo sapiens

<220>
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<222> (1)..(1755)

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gat gca cac aag agt gag gtt gct cat cggtt aaa gat ttggaa gaa 48
Asp Ala His Lys Ser Glu Val Ala His Arg Phe Lys Asp Leu Gly Glu
1 5 10 15

gaa aat ttc aaa gcc ttgttg att gcc ttt gct cag tat ctt cag 96
Glu Asn Phe Lys Ala Leu Val Leu Ile Ala Phe Ala Gln Tyr Leu Gln
20 25 30

cag tgt cca ttt gaa gat cat gta aaa ttatgtg aat gaa gta act gaa 144
Gln Cys Pro Phe Glu Asp His Val Lys Leu Val Asn Glu Val Thr Glu
35 40 45

ttt gca aaa aca tgt gtt gct gat gag tca gct gaa aat tgt gac aaa 192
Phe Ala Lys Thr Cys Val Ala Asp Glu Ser Ala Glu Asn Cys Asp Lys
50 55 60

tca ctt cat acc ctt ttt gga gac aaa ttatgcaca gtt gca act ctt 240
Ser Leu His Thr Leu Phe Gly Asp Lys Leu Cys Thr Val Ala Thr Leu
65 70 75 80

cgt gaa acc tat ggt gaa atg gct gac tgc tgt gca aaa caa gaa cct 288
Arg Glu Thr Tyr Gly Glu Met Ala Asp Cys Cys Ala Lys Gln Glu Pro
85 90 95

gag aga aat gaa tgc ttgttgc caa cac aaa gat gac aac cca aac ctc 336
Glu Arg Asn Glu Cys Phe Leu Gln His Lys Asp Asp Asn Pro Asn Leu
100 105 110

ccc cga ttgtgtg aga cca gag gtt gat gtg atg tgc act gct ttcat 384
Pro Arg Leu Val Arg Pro Glu Val Asp Val Met Cys Thr Ala Phe His

115	120	125	
gac aat gaa gag aca ttt ttg aaa aaa tac tta tat gaa att gcc aga Asp Asn Glu Glu Thr Phe Leu Lys Lys Tyr Leu Tyr Glu Ile Ala Arg 130	135	140	432
aga cat cct tac ttt tat gcc ccg gaa ctc ctt ttc ttt gct aaa agg Arg His Pro Tyr Phe Tyr Ala Pro Glu Leu Leu Phe Phe Ala Lys Arg 145	150	155	480
tat aaa gct gct ttt aca gaa tgt tgc caa gct gct gat aaa gct gcc Tyr Lys Ala Ala Phe Thr Glu Cys Cys Gln Ala Ala Asp Lys Ala Ala 165	170	175	528
tgc ctg ttg cca aag ctc gat gaa ctt cg ^g gat gaa ggg aag gct tcg Cys Leu Leu Pro Lys Leu Asp Glu Leu Arg Asp Glu Gly Lys Ala Ser 180	185	190	576
tct gcc aaa cag aga ctc aaa tgt gcc agt ctc caa aaa ttt gga gaa Ser Ala Lys Gln Arg Leu Lys Cys Ala Ser Leu Gln Lys Phe Gly Glu 195	200	205	624
aga gct ttc aaa gca tgg gca gtg gct cgc ctg agc cag aga ttt ccc Arg Ala Phe Lys Ala Trp Ala Val Ala Arg Leu Ser Gln Arg Phe Pro 210	215	220	672
aaa gct gag ttt gca gaa gtt tcc aag tta gtg aca gat ctt acc aaa Lys Ala Glu Phe Ala Glu Val Ser Lys Leu Val Thr Asp Leu Thr Lys 225	230	235	720
gtc cac acg gaa tgc tgc cat gga gat ctg ctt gaa tgt gct gat gac Val His Thr Glu Cys Cys His Gly Asp Leu Leu Glu Cys Ala Asp Asp 245	250	255	768
agg gcg gac ctt gcc aag tat atc tgt gaa aat cag gat tcg atc tcc Arg Ala Asp Leu Ala Lys Tyr Ile Cys Glu Asn Gln Asp Ser Ile Ser 260	265	270	816
agt aaa ctg aag gaa tgc tgt gaa aaa cct ctg ttg gaa aaa tcc cac Ser Lys Leu Lys Glu Cys Cys Glu Lys Pro Leu Leu Glu Lys Ser His 275	280	285	864
tgc att gcc gaa gtg gaa aat gat gag atg cct gct gac ttg cct tca Cys Ile Ala Glu Val Glu Asn Asp Glu Met Pro Ala Asp Leu Pro Ser 290	295	300	912
tta gct gct gat ttt gtt gaa agt aag gat gtt tgc aaa aac tat gct Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala 305	310	315	960
gag gca aag gat gtc ttc ctg ggc atg ttt ttg tat gaa tat gca aga Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg 325	330	335	1008
agg cat cct gat tac tct gtc gtg ctg ctg aga ctt gcc aag aca Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr 340	345	350	1056
tat gaa acc act cta gag aag tgc tgt gcc gct gca gat cct cat gaa Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu			1104

355	360	365	
tgc tat gcc aaa gtg ttc gat gaa ttt aaa cct ctt gtg gaa gag cct Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro 370	375	380	1152
cag aat tta atc aaa caa aac tgt gag ctt ttt gag cag ctt gga gag Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu 385	390	395	1200
tac aaa ttc cag aat gcg cta tta gtt cgt tac acc aag aaa gta ccc Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro 405	410	415	1248
caa gtg tca act cca act ctt gta gag gtc tca aga aac cta gga aaa Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys 420	425	430	1296
gtg ggc agc aaa tgt tgt aaa cat cct gaa gca aaa aga atg ccc tgt Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys 435	440	445	1344
gca gaa gac tat cta tcc gtg gtc ctg aac cag tta tgt gtg ttg cat Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His 450	455	460	1392
gag aaa acg cca gta agt gac aga gtc aca aaa tgc tgc aca gag tcc Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser 465	470	475	1440
ttg gtg aac agg cga cca tgc ttt tca gct ctg gaa gtc gat gaa aca Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr 485	490	495	1488
tac gtt ccc aaa gag ttt aat gct gaa aca ttc acc ttc cat gca gat Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp 500	505	510	1536
ata tgc aca ctt tct gag aag gag aga caa atc aag aaa caa act gca Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala 515	520	525	1584
ctt gtt gag ctt gtg aaa cac aag ccc aag gca aca aaa gag caa ctg Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu 530	535	540	1632
aaa gct gtt atg gat gat ttc gca gct ttt gta gag aag tgc tgc aag Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys 545	550	555	1680
gct gac gat aag gag acc tgc ttt gcc gag gag ggt aaa aaa ctt gtt Ala Asp Asp Lys Glu Thr Cys Phe Ala Glu Glu Gly Lys Lys Leu Val 565	570	575	1728
gct gca agt caa gct gcc tta ggc tta taacatctac atttaaaaagc atctcag Ala Ala Ser Gln Ala Ala Leu Gly Leu 580	585		1782

<211> 585

<212> PRT

<213> Homo Sapiens

<400> 18

Asp	Ala	His	Lys	Ser	Glu	Val	Ala	His	Arg	Phe	Lys	Asp	Leu	Gly	Glu
1				5				10					15		

Glu	Asn	Phe	Lys	Ala	Leu	Val	Leu	Ile	Ala	Phe	Ala	Gln	Tyr	Leu	Gln
				20				25				30			

Gln	Cys	Pro	Phe	Glu	Asp	His	Val	Lys	Leu	Val	Asn	Glu	Val	Thr	Glu
					35			40			45				

Phe	Ala	Lys	Thr	Cys	Val	Ala	Asp	Glu	Ser	Ala	Glu	Asn	Cys	Asp	Lys
					50			55		60					

Ser	Leu	His	Thr	Leu	Phe	Gly	Asp	Lys	Leu	Cys	Thr	Val	Ala	Thr	Leu
					65			70		75		80			

Arg	Glu	Thr	Tyr	Gly	Glu	Met	Ala	Asp	Cys	Cys	Ala	Lys	Gln	Glu	Pro
					85				90		95				

Glu	Arg	Asn	Glu	Cys	Phe	Leu	Gln	His	Lys	Asp	Asp	Asn	Pro	Asn	Leu
					100			105				110			

Pro	Arg	Leu	Val	Arg	Pro	Glu	Val	Asp	Val	Met	Cys	Thr	Ala	Phe	His
					115			120			125				

Asp	Asn	Glu	Glu	Thr	Phe	Leu	Lys	Lys	Tyr	Leu	Tyr	Glu	Ile	Ala	Arg
					130			135		140					

Arg	His	Pro	Tyr	Phe	Tyr	Ala	Pro	Glu	Leu	Leu	Phe	Phe	Ala	Lys	Arg
					145			150		155		160			

Tyr	Lys	Ala	Ala	Phe	Thr	Glu	Cys	Cys	Gln	Ala	Ala	Asp	Lys	Ala	Ala
					165			170		175					

Cys	Leu	Leu	Pro	Lys	Leu	Asp	Glu	Leu	Arg	Asp	Glu	Gly	Lys	Ala	Ser
					180			185			190				

Ser	Ala	Lys	Gln	Arg	Leu	Lys	Cys	Ala	Ser	Leu	Gln	Lys	Phe	Gly	Glu
					195			200		205					

Arg	Ala	Phe	Lys	Ala	Trp	Ala	Val	Ala	Arg	Leu	Ser	Gln	Arg	Phe	Pro
					210			215		220					

Lys	Ala	Glu	Phe	Ala	Glu	Val	Ser	Lys	Leu	Val	Thr	Asp	Leu	Thr	Lys
					225			230		235		240			

Val	His	Thr	Glu	Cys	Cys	His	Gly	Asp	Leu	Leu	Glu	Cys	Ala	Asp	Asp
					245			250		255					

Arg	Ala	Asp	Leu	Ala	Lys	Tyr	Ile	Cys	Glu	Asn	Gln	Asp	Ser	Ile	Ser
					260			265		270					

Ser	Lys	Leu	Lys	Glu	Cys	Cys	Glu	Lys	Pro	Leu	Leu	Glu	Lys	Ser	His
					275			280		285					

Cys	Ile	Ala	Glu	Val	Glu	Asn	Asp	Glu	Met	Pro	Ala	Asp	Leu	Pro	Ser
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290	295	300
Leu Ala Ala Asp Phe Val Glu Ser Lys Asp Val Cys Lys Asn Tyr Ala		
305	310	315
Glu Ala Lys Asp Val Phe Leu Gly Met Phe Leu Tyr Glu Tyr Ala Arg		
325	330	335
Arg His Pro Asp Tyr Ser Val Val Leu Leu Leu Arg Leu Ala Lys Thr		
340	345	350
Tyr Glu Thr Thr Leu Glu Lys Cys Cys Ala Ala Ala Asp Pro His Glu		
355	360	365
Cys Tyr Ala Lys Val Phe Asp Glu Phe Lys Pro Leu Val Glu Glu Pro		
370	375	380
Gln Asn Leu Ile Lys Gln Asn Cys Glu Leu Phe Glu Gln Leu Gly Glu		
385	390	395
Tyr Lys Phe Gln Asn Ala Leu Leu Val Arg Tyr Thr Lys Lys Val Pro		
405	410	415
Gln Val Ser Thr Pro Thr Leu Val Glu Val Ser Arg Asn Leu Gly Lys		
420	425	430
Val Gly Ser Lys Cys Cys Lys His Pro Glu Ala Lys Arg Met Pro Cys		
435	440	445
Ala Glu Asp Tyr Leu Ser Val Val Leu Asn Gln Leu Cys Val Leu His		
450	455	460
Glu Lys Thr Pro Val Ser Asp Arg Val Thr Lys Cys Cys Thr Glu Ser		
465	470	475
Leu Val Asn Arg Arg Pro Cys Phe Ser Ala Leu Glu Val Asp Glu Thr		
485	490	495
Tyr Val Pro Lys Glu Phe Asn Ala Glu Thr Phe Thr Phe His Ala Asp		
500	505	510
Ile Cys Thr Leu Ser Glu Lys Glu Arg Gln Ile Lys Lys Gln Thr Ala		
515	520	525
Leu Val Glu Leu Val Lys His Lys Pro Lys Ala Thr Lys Glu Gln Leu		
530	535	540
Lys Ala Val Met Asp Asp Phe Ala Ala Phe Val Glu Lys Cys Cys Lys		
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1 5 10 15

Tyr Ser Arg Ser Leu Asp Lys Arg
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<210> 30
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<210> 67
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<400> 67
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<212> DNA
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<220>
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23

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<400> 71
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23

<210> 72
<211> 15
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<213> Artificial Sequence

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1															15
	5														
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							20								
<210> 74															
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<212> PRT															
<213> Homo sapiens															
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1															15
	5														
Ala	Gly	Ser	Leu	Gln	Gly	Lys	Pro	Leu	Gln	Ser	Trp	Gly	Arg	Gly	Ser
															30
	20							25							
Ala	Gly	Gly	Asn	Ala	His	Ser	Pro	Leu	Gly	Val	Pro	Gly	Gly	Gly	Leu
															45
	35							40							
Pro	Glu	His	Thr	Phe	Asn	Leu	Lys	Met	Phe	Leu	Glu	Asn	Val	Lys	Val
															60
	50							55							
Asp	Phe	Leu	Arg	Ser	Leu	Asn	Leu	Ser	Gly	Val	Pro	Ser	Gln	Asp	Lys
															80
	65							70							
Thr	Arg	Val	Glu	Pro	Pro	Gln	Tyr	Met	Ile	Asp	Leu	Tyr	Asn	Arg	Tyr
															95
	85							90							
Thr	Ser	Asp	Lys	Ser	Thr	Thr	Pro	Ala	Ser	Asn	Ile	Val	Arg	Ser	Phe
															110
	100							105							
Ser	Met	Glu	Asp	Ala	Ile	Ser	Ile	Thr	Ala	Thr	Glu	Asp	Phe	Pro	Phe
															125
	115							120							
Gln	Lys	His	Ile	Leu	Leu	Phe	Asn	Ile	Ser	Ile	Pro	Arg	His	Glu	Gln
															140
	130							135							
Ile	Thr	Arg	Ala	Glu	Leu	Arg	Leu	Tyr	Val	Ser	Cys	Gln	Asn	His	Val
															160
	145							150							
Asp	Pro	Ser	His	Asp	Leu	Lys	Gly	Ser	Val	Val	Ile	Tyr	Asp	Val	Leu
															175
	165							170							
Asp	Gly	Thr	Asp	Ala	Trp	Asp	Ser	Ala	Thr	Glu	Thr	Lys	Thr	Phe	Leu
															190
	180							185							
Val	Ser	Gln	Asp	Ile	Gln	Asp	Glu	Gly	Trp	Glu	Thr	Leu	Glu	Val	Ser
															205
	195							200							
Ser	Ala	Val	Lys	Arg	Trp	Val	Arg	Ser	Ser	Thr	Lys	Ser	Lys	Asn	

210	215	220
Lys Leu Glu Val Thr Val Glu Ser His Arg Lys Gly Cys Asp Thr Leu		
225	230	235
Asp Ile Ser Val Pro Pro Gly Ser Arg Asn Leu Pro Phe Phe Val Val		
245	250	255
Phe Ser Asn Asp His Ser Ser Gly Thr Lys Glu Thr Arg Leu Glu Leu		
260	265	270
Arg Glu Met Ile Ser His Glu Gln Glu Ser Val Leu Lys Lys Leu Ser		
275	280	285
Lys Asp Gly Ser Thr Glu Ala Gly Glu Ser Ser His Glu Glu Asp Thr		
290	295	300
Asp Gly His Val Ala Ala Gly Ser Thr Leu Ala Arg Arg Lys Arg Ser		
305	310	315
Ala Gly Ala Gly Ser His Cys Gln Lys Thr Ser Leu Arg Val Asn Phe		
325	330	335
Glu Asp Ile Gly Trp Asp Ser Trp Ile Ile Ala Pro Lys Glu Tyr Glu		
340	345	350
Ala Tyr Glu Cys Lys Gly Gly Cys Phe Phe Pro Leu Ala Asp Asp Val		
355	360	365
Thr Pro Thr Lys His Ala Ile Val Gln Thr Leu Val His Leu Lys Phe		
370	375	380
Pro Thr Lys Val Gly Lys Ala Cys Cys Val Pro Thr Lys Leu Ser Pro		
385	390	395
Ile Ser Val Leu Tyr Lys Asp Asp Met Gly Val Pro Thr Leu Lys Tyr		
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15		
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20	25	30
Val Ile Thr Asp Glu Asn Trp Arg Glu Leu Leu Glu Gly Asp Trp Met		
35	40	45
Ile Glu Phe Tyr Ala Pro Trp Cys Pro Ala Cys Gln Asn Leu Gln Pro		
50	55	60
Glu Trp Glu Ser Phe Ala Glu Trp Gly Glu Asp Leu Glu Val Asn Ile		

65	70	75	80
Ala Lys Val Asp Val Thr Glu Gln Pro Gly Leu Ser Gly Arg Phe Ile			
85 90 95			
Ile Thr Ala Leu Pro Thr Ile Tyr His Cys Lys Asp Gly Glu Phe Arg			
100 105 110			
Arg Tyr Gln Gly Pro Arg Thr Lys Lys Asp Phe Ile Asn Phe Ile Ser			
115 120 125			
Asp Lys Glu Trp Lys Ser Ile Glu Pro Val Ser Ser Trp Phe Gly Pro			
130 135 140			
Gly Ser Val Leu Met Ser Ser Met Ser Ala Leu Phe Gln Leu Ser Met			
145 150 155 160			
Trp Ile Arg Thr Cys His Asn Tyr Phe Ile Glu Asp Leu Gly Leu Pro			
165 170 175			
Val Trp Gly Ser Tyr Thr Val Phe Ala Leu Ala Thr Leu Phe Ser Gly			
180 185 190			
Leu Leu Leu Gly Leu Cys Met Ile Phe Val Ala Asp Cys Leu Cys Pro			
195 200 205			
Ser Lys Arg Arg Arg Pro Gln Pro Tyr Pro Tyr Pro Ser Lys Lys Leu			
210 215 220			
Leu Ser Glu Ser Ala Gln Pro Leu Lys Lys Val Glu Glu Glu Gln Glu			
225 230 235 240			
Ala Asp Glu Glu Asp Val Ser Glu Glu Glu Ala Glu Ser Lys Glu Gly			
245 250 255			
Thr Asn Lys Asp Phe Pro Gln Asn Ala Ile Arg Gln Arg Ser Leu Gly			
260 265 270			
Pro Ser Leu Ala Thr Asp Lys Ser			
275 280			
<210> 76			
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<400> 76			
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1 5 10 15			
Phe Arg Leu Pro Arg Lys Trp Gly Trp Arg Thr Glu Ala Thr Ala Pro			
20 25 30			
His Ala Pro Val Pro Gln Ser Ile Cys Pro Arg Tyr Thr Ser Pro Cys			
35 40 45			
Ala Pro His Asp Cys Gly Ser Gln Thr Val Gln Gly Asn Ser Leu Ser			
50 55 60			
Leu Phe Tyr Thr Leu Ser His Lys Ala Pro Gln Leu Pro His Arg Val			

65	70	75	80
----	----	----	----

Pro Ala Pro Leu Phe Cys Lys Tyr Val Lys Arg Lys Lys Cys Lys Arg	85	90	95
---	----	----	----

Trp Ser Leu Gly Trp Ser Ser Ser Leu Gln Leu Arg Leu Leu Thr Met	100	105	110
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<210> 77

<211> 346

<212> PRT

<213> Homo sapiens

<400> 77

Met Asp Pro Ala Arg Lys Ala Gly Ala Gln Ala Met Ile Trp Thr Ala	1	5	10	15
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Gly Trp Leu Leu Leu Leu Leu Arg Gly Gly Ala Gln Ala Leu Glu	20	25	30
---	----	----	----

Cys Tyr Ser Cys Val Gln Lys Ala Asp Asp Gly Cys Ser Pro Asn Lys	35	40	45
---	----	----	----

Met Lys Thr Val Lys Cys Ala Pro Gly Val Asp Val Cys Thr Glu Ala	50	55	60
---	----	----	----

Val Gly Ala Val Glu Thr Ile His Gly Gln Phe Ser Leu Ala Val Arg	65	70	75	80
---	----	----	----	----

Gly Cys Gly Ser Gly Leu Pro Gly Lys Asn Asp Arg Gly Leu Asp Leu	85	90	95
---	----	----	----

His Gly Leu Leu Ala Phe Ile Gln Leu Gln Gln Cys Ala Gln Asp Arg	100	105	110
---	-----	-----	-----

Cys Asn Ala Lys Leu Asn Leu Thr Ser Arg Ala Leu Asp Pro Ala Gly	115	120	125
---	-----	-----	-----

Asn Glu Ser Ala Tyr Pro Pro Asn Gly Val Glu Cys Tyr Ser Cys Val	130	135	140
---	-----	-----	-----

Gly Leu Ser Arg Glu Ala Cys Gln Gly Thr Ser Pro Pro Val Val Ser	145	150	155	160
---	-----	-----	-----	-----

Cys Tyr Asn Ala Ser Asp His Val Tyr Lys Gly Cys Phe Asp Gly Asn	165	170	175
---	-----	-----	-----

Val Thr Leu Thr Ala Ala Asn Val Thr Val Ser Leu Pro Val Arg Gly	180	185	190
---	-----	-----	-----

Cys Val Gln Asp Glu Phe Cys Thr Arg Asp Gly Val Thr Gly Pro Gly	195	200	205
---	-----	-----	-----

Phe Thr Leu Ser Gly Ser Cys Cys Gln Gly Ser Arg Cys Asn Ser Asp	210	215	220
---	-----	-----	-----

Leu Arg Asn Lys Thr Tyr Phe Ser Pro Arg Ile Pro Pro Leu Val Arg	225	230	235	240
---	-----	-----	-----	-----

Leu Pro Pro Pro Glu Pro Thr Thr Val Ala Ser Thr Thr Ser Val Thr

245	250	255
Thr Ser Thr Ser Ala Pro Val Arg Pro Thr Ser Thr Thr Lys Pro Met		
260	265	270
Pro Ala Pro Thr Ser Gln Thr Pro Arg Gln Gly Val Glu His Glu Ala		
275	280	285
Ser Arg Asp Glu Glu Pro Arg Leu Thr Gly Gly Ala Ala Gly His Gln		
290	295	300
Asp Arg Ser Asn Ser Gly Gln Tyr Pro Ala Lys Gly Gly Pro Gln Gln		
305	310	315
Pro His Asn Lys Gly Cys Val Ala Pro Thr Ala Gly Leu Ala Ala Leu		
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<213> Homo sapiens		
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15		
Asp Glu Asp Ser Met Asp Ile Pro Leu Asp Leu Ser Ser Ser Ala Gly		
20	25	30
Ser Gly Lys Arg Arg Arg Arg Gly Asn Leu Pro Lys Glu Ser Val Gln		
35	40	45
Ile Leu Arg Asp Trp Leu Tyr Glu His Arg Tyr Asn Ala Tyr Pro Ser		
50	55	60
Glu Gln Glu Lys Ala Leu Leu Ser Gln Gln Thr His Leu Ser Thr Leu		
65	70	75
80		
Gln Val Cys Asn Trp Phe Ile Asn Ala Arg Arg Arg Leu Leu Pro Asp		
85	90	95
Met Leu Arg Lys Asp Gly Lys Asp Pro Asn Gln Phe Thr Ile Ser Arg		
100	105	110
Arg Gly Ala Lys Ile Ser Glu Thr Ser Ser Val Glu Ser Val Met Gly		
115	120	125
Ile Lys Asn Phe Met Pro Ala Leu Glu Glu Thr Pro Phe His Ser Cys		
130	135	140
Thr Ala Gly Pro Asn Pro Thr Leu Gly Arg Pro Leu Ser Pro Lys Pro		
145	150	155
160		
Ser Ser Pro Gly Ser Val Leu Ala Arg Pro Ser Val Ile Cys His Thr		
165	170	175
Thr Val Thr Ala Leu Lys Asp Val Pro Phe Ser Leu Cys Gln Ser Val		

180	185	190
Gly Val Gly Gln Asn Thr Asp Ile Gln Gln Ile Ala Ala Lys Asn Phe		
195	200	205
Thr Asp Thr Ser Leu Met Tyr Pro Glu Asp Thr Cys Lys Ser Gly Pro		
210	215	220
Ser Thr Asn Thr Gln Ser Gly Leu Phe Asn Thr Pro Pro Pro Thr Pro		
225	230	235
Pro Asp Leu Asn Gln Asp Phe Ser Gly Phe Gln Leu Leu Val Asp Val		
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<213> Homo sapiens		
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35	40	45
Ile Thr Ala Leu Arg Val Arg Val Asn Thr Tyr Tyr Ile Val Gly Leu		
50	55	60
Gln Val Arg Tyr Gly Lys Val Trp Ser Asp Tyr Val Gly Gly Arg Asn		
65	70	75
80		
Gly Asp Leu Glu Glu Ile Phe Leu His Pro Gly Glu Ser Val Ile Gln		
85	90	95
Val Ser Gly Lys Tyr Lys Trp Tyr Leu Lys Lys Leu Val Phe Val Thr		
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Asp Lys Gly Arg Tyr Leu Ser Phe Gly Lys Asp Ser Gly Thr Ser Phe		
115	120	125
Asn Ala Val Pro Leu His Pro Asn Thr Val Leu Arg Phe Ile Ser Gly		
130	135	140
Arg Ser Gly Ser Leu Ile Asp Ala Ile Gly Leu His Trp Asp Val Tyr		
145	150	155
160		
Pro Thr Ser Cys Ser Arg Cys		
165		

**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL**

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 96, line 30.

B. IDENTIFICATION OF DEPOSIT

Further deposits are identified on an additional sheet

Name of depositary institution: American Type Culture Collection

Address of depositary institution (*including postal code and country*)

10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit

11 April 2001

Accession Number

PTA-3276

C. ADDITIONAL INDICATIONS (Leave blank if not applicable)

This information is continued on an additional sheet

D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)

Europe

In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

Continued on additional sheets

E. SEPARATE FURNISHING OF INDICATIONS (Leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g. "Accession Number of Deposit")

	For receiving Office use only			For International Bureau use only	
<input type="checkbox"/> This sheet was received with the international application				<input type="checkbox"/> This sheet was received by the International Bureau on	
Authorized officer				Authorized officer	

ATCC Deposit No.: PTA-3276

CANADA

The applicant requests that, until either a Canadian patent has been issued on the basis of an application or the application has been refused, or is abandoned and no longer subject to reinstatement, or is withdrawn, the Commissioner of Patents only authorizes the furnishing of a sample of the deposited biological material referred to in the application to an independent expert nominated by the Commissioner, the applicant must, by a written statement, inform the International Bureau accordingly before completion of technical preparations for publication of the international application.

NORWAY

The applicant hereby requests that the application has been laid open to public inspection (by the Norwegian Patent Office), or has been finally decided upon by the Norwegian Patent Office without having been laid open inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Norwegian Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Norwegian Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on the list of recognized experts drawn up by the Norwegian Patent Office or any person approved by the applicant in the individual case.

AUSTRALIA

The applicant hereby gives notice that the furnishing of a sample of a microorganism shall only be effected prior to the grant of a patent, or prior to the lapsing, refusal or withdrawal of the application, to a person who is a skilled addressee without an interest in the invention (Regulation 3.25(3) of the Australian Patents Regulations).

FINLAND

The applicant hereby requests that, until the application has been laid open to public inspection (by the National Board of Patents and Regulations), or has been finally decided upon by the National Board of Patents and Registration without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art.

UNITED KINGDOM

The applicant hereby requests that the furnishing of a sample of a microorganism shall only be made available to an expert. The request to this effect must be filed by the applicant with the International Bureau before the completion of the technical preparations for the international publication of the application.

ATCC Deposit No.: PTA-3276**DENMARK**

The applicant hereby requests that, until the application has been laid open to public inspection (by the Danish Patent Office), or has been finally decided upon by the Danish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the Danish Patent Office not later than at the time when the application is made available to the public under Sections 22 and 33(3) of the Danish Patents Act. If such a request has been filed by the applicant, any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Danish Patent Office or any person by the applicant in the individual case.

SWEDEN

The applicant hereby requests that, until the application has been laid open to public inspection (by the Swedish Patent Office), or has been finally decided upon by the Swedish Patent Office without having been laid open to public inspection, the furnishing of a sample shall only be effected to an expert in the art. The request to this effect shall be filed by the applicant with the International Bureau before the expiration of 16 months from the priority date (preferably on the Form PCT/RO/134 reproduced in annex Z of Volume I of the PCT Applicant's Guide). If such a request has been filed by the applicant any request made by a third party for the furnishing of a sample shall indicate the expert to be used. That expert may be any person entered on a list of recognized experts drawn up by the Swedish Patent Office or any person approved by a applicant in the individual case.

NETHERLANDS

The applicant hereby requests that until the date of a grant of a Netherlands patent or until the date on which the application is refused or withdrawn or lapsed, the microorganism shall be made available as provided in the 31F(1) of the Patent Rules only by the issue of a sample to an expert. The request to this effect must be furnished by the applicant with the Netherlands Industrial Property Office before the date on which the application is made available to the public under Section 22C or Section 25 of the Patents Act of the Kingdom of the Netherlands, whichever of the two dates occurs earlier.

**INDICATIONS RELATING TO A DEPOSITED MICROORGANISM
OR OTHER BIOLOGICAL MATERIAL**

(PCT Rule 13bis)

A. The indications made below relate to the deposited microorganism or other biological material referred to in the description on page 96, line 30.

B. IDENTIFICATION OF DEPOSIT

Further deposits are identified on an additional sheet

Name of depositary institution: American Type Culture Collection

Address of depositary institution (*including postal code and country*)

10801 University Boulevard
Manassas, Virginia 20110-2209
United States of America

Date of deposit 11 April 2001	Accession Number PTA-3277
----------------------------------	------------------------------

C. ADDITIONAL INDICATIONS (leave blank if not applicable)	This information is continued on an additional sheet <input type="checkbox"/>
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D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (*if the indications are not for all designated States*)

Europe

In respect of those designations in which a European Patent is sought a sample of the deposited microorganism will be made available until the publication of the mention of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample (Rule 28(4) EPC).

Continued on additional sheets

E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later (*specify the general nature of the indications e.g. "Accession Number of Deposit"*)

	For receiving Office use only			For International Bureau use only	
<input type="checkbox"/> This sheet was received with the international application		<input type="checkbox"/> This sheet was received by the International Bureau on			
Authorized officer		Authorized officer			

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/11941

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 37/02; C12N 15/00

US CL : 530/350; 435/7.1

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/350; 435/7.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
EST, GENEMBL, AGENESEQ

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN: MEDLINE, BIOSIS, USPAT, JAPIO, HCAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95/23857 A1 (DELTA BIOTECHNOLOGY LIMITED) 08 September 1995 (08.09.95), see entire document.	1-9, 15-19
X	EP 0 322 094 A1 (DELTA BIOTECHNOLOGY LIMITED) 28 June 1989 (28.06.89), see entire document.	1-9, 15-19

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search
18 JULY 2001

Date of mailing of the international search report

14 AUG 2001

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-9290Authorized officer
HOPE ROBINSON
Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/11991

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. **Claims Nos.:**
because they relate to subject matter not required to be searched by this Authority, namely:

2. **Claims Nos.:**
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. **Claims Nos.: 10-14, 20-32 and 34-36**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-9, 15-19 (FGF-7)

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/11991

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

Groups 1-58, claim(s) 1-9, 15-19, all in part, drawn to a therapeutic protein X, wherein X correlates to those listed in the Table on page 11 of the description. If any of Groups 1-58 are elected the claims will only be examined in-so-far-as it pertains to the elected protein X. For example,
If Group 1 is elected, this correlates to Therapeutic Protein X:FGF-7.
Group 2, all partially as in Group 1, concerning Therapeutic Protein X: Kertainocyte growth factor 2.
Group 3, all partially as in Group 1, concerning Therapeutic Protein X: C-C Chemokine Receptor 5.
Group 4, all partially as in Group 1, concerning Therapeutic Protein X: Cathepsin K.
Group 5, all partially as in Group 1, concerning Therapeutic Protein X: MPIF-1 (Myeloid Progenitor Inhibitory Factor).
Group 6, all partially as in Group 1, concerning Therapeutic Protein X: VEGF.
Group 7, all partially as in Group 1, concerning Therapeutic Protein X: VEGF-2.
Group 8, all partially as in Group 1, concerning Therapeutic Protein X: BLyS.
Group 9, all partially as in Group 1, concerning Therapeutic Protein X: KD1.
Group 10, all partially as in Group 1, concerning Therapeutic Protein X: TMP-1.
Group 11, all partially as in Group 1, concerning Therapeutic Protein X: TMP-2.
Group 12, all partially as in Group 1, concerning Therapeutic Protein X: TMP-3.
Group 13, all partially as in Group 1, concerning Therapeutic Protein X: TMP-4.
Group 14, all partially as in Group 1, concerning Therapeutic Protein X: Connective Tissue Growth Factor Protein.
Group 15, all partially as in Group 1, concerning Therapeutic Protein X:CTGF-2.
Group 16, all partially as in Group 1, concerning Therapeutic Protein X: Connective Tissue Growth Factor 4.
Group 17, all partially as in Group 1, concerning Therapeutic Protein X: Human T-cell Lymphoma-lipoprotein associated phospholipase-A2.
Group 18, all partially as in Group 1, concerning Therapeutic Protein X: VEGI.
Group 19, all partially as in Group 1, concerning Therapeutic Protein X: AIM-1.
Group 20, all partially as in Group 1, concerning Therapeutic Protein X: TNF-delta.
Group 21, all partially as in Group 1, concerning Therapeutic Protein X: TNF-epsilon.
Group 22, all partially as in Group 1, concerning Therapeutic Protein X: AIM-2.
Group 23, all partially as in Group 1, concerning Therapeutic Protein X: Endokine.
Group 24, all partially as in Group 1, concerning Therapeutic Protein X: TR1.
Group 25, all partially as in Group 1, concerning Therapeutic Protein X:TR2.
Group 26, all partially as in Group 1, concerning Therapeutic Protein X: DR3.
Group 27, all partially as in Group 1, concerning Therapeutic Protein X: TR4.
Group 28, all partially as in Group 1, concerning Therapeutic Protein X: 4-1BBSv receptor.
Group 29, all partially as in Group 1, concerning Therapeutic Protein X: OPG.
Group 30, all partially as in Group 1, concerning Therapeutic Protein X: FasL.
Group 31, all partially as in Group 1, concerning Therapeutic Protein X: Fas.
Group 32, all partially as in Group 1, concerning Therapeutic Protein X: TR5.
Group 33, all partially as in Group 1, concerning Therapeutic Protein X: TR6.
Group 34, all partially as in Group 1, concerning Therapeutic Protein X: DR5.
Group 35, all partially as in Group 1, concerning Therapeutic Protein X: TR8.
Group 36, all partially as in Group 1, concerning Therapeutic Protein X: TR9.
Group 37, all partially as in Group 1, concerning Therapeutic Protein X: TR10.
Group 38, all partially as in Group 1, concerning Therapeutic Protein X: TR11.
Group 39, all partially as in Group 1, concerning Therapeutic Protein X: TR12.
Group 40, all partially as in Group 1, concerning Therapeutic Protein X: TR13.
Group 41, all partially as in Group 1, concerning Therapeutic Protein X: TR14.
Group 42, all partially as in Group 1, concerning Therapeutic Protein X: TR16.
Group 43, all partially as in Group 1, concerning Therapeutic Protein X: HLDOU18.
Group 44, all partially as in Group 1, concerning Therapeutic Protein X: HSDSB09.
Group 45, all partially as in Group 1, concerning Therapeutic Protein X: HDPBQ71.
Group 46, all partially as in Group 1, concerning Therapeutic Protein X: HAGDG59.
Group 47, all partially as in Group 1, concerning Therapeutic Protein X: HCHNF25.
Group 48, all partially as in Group 1, concerning Therapeutic Protein X: HKACD58.
Group 49, all partially as in Group 1, concerning Therapeutic Protein X: HWACB86.
Group 50, all partially as in Group 1, concerning Therapeutic Protein X: HFTCF50.
Group 51, all partially as in Group 1, concerning Therapeutic Protein X: HRDFD27.
Group 52, all partially as in Group 1, concerning Therapeutic Protein X: HCEGG08.
Group 53, all partially as in Group 1, concerning Therapeutic Protein X: HKACI79.

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Group 54, all partially as in Group 1, concerning Therapeutic Protein X: HWHGZ51.
Group 55, all partially as in Group 1, concerning Therapeutic Protein X: HDTAI21.
Group 56, all partially as in Group 1, concerning Therapeutic Protein X: HCNCA73.
Group 57, all partially as in Group 1, concerning Therapeutic Protein X: HNHFE71.
Group 58, all partially as in Group 1, concerning Therapeutic Protein X: HLWCF05.

Group 59-117, claim(s) 38, drawn to a method of extending shelf life of Therapeutic Protein X, wherein Therapeutic Protein X can be any of the proteins listed in the Table on page 11 of the description. If any of Groups 59-117 are elected the claim will only be examined in-so-far-as it pertains to the elected Protein X.

The inventions listed as Groups 1-118 do not relate to a single inventive concept under PCT Rule 18.1 because, under PCT Rule 18.2, they lack the same or corresponding special technical features for the following reasons: because the technical feature linking groups 1-118 is not special because Inventions 1-58 do not avoid the prior art as Delta Biotechnology Limited, (EP 322094, June 28, 1989) teaches the claimed sequence. Thus, the invention does not relate to a single inventive concept and is not a contribution over the prior art.